

ECOLOGICAL COMMUNITY ASSEMBLY IN THE FACE OF ANTHROPOGENIC ENVIRONMENTAL CHANGES

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ECOLOGICAL COMMUNITY ASSEMBLY IN THE FACE OF ANTHROPOGENIC ENVIRONMENTAL CHANGES

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SUMMARY

Anthropogenic environmental changes, such as increased nitrogen (N) deposition, changes in precipitation regimes, and habitat loss and fragmentation, are known to affect Earth's ecosystems. Understanding mechanisms regulating the assembly of ecological communities in the face of anthropogenic environmental changes is one of the primary goals of contemporary ecology. In this dissertation, I present four studies addressing questions on community assembly under anthropogenic environmental changes. Two field experimental studies were conducted in a semi-arid grassland to examine how anthropogenic environmental changes, in the form of resource addition, influence phylogenetic alpha- (Chapter 1) and beta-diversity (Chapter 2) dynamics of the impacted communities. One laboratory experimental study (Chapter 3) used bacterivorous ciliated protists as model organisms to explore the impacts of losing a keystone local community on metacommunity biodiversity and ecosystem functions. One field observational study (Chapter 4) was conducted in a fragmented subtropical forest to investigate the relative importance of deterministic and stochastic processes in shaping phyllosphere microbial communities in the context of habitat fragmentation.

The first chapter describes an experimental study conducted in a temperate steppe in northern China to explore the effects of N enrichment and increased precipitation on plant community phylogenetic structure. This study demonstrated that N and water addition influenced different aspects of plant community structure in the grassland ecosystem. Water addition increased plant species richness by preventing species extinction and facilitating species colonization, without altering community phylogenetic

structure. In contrast, N addition did not alter species richness, but promoted colonization of species distantly related to the resident species, resulting in changes in community phylogenetic structure (from being neutral to overdispersion).

Building on work described in the first chapter, I explored in the second chapter how N and precipitation amendments affect the taxonomic and phylogenetic β -diversity of plant communities in the temperate steppe ecosystem in northern China. Both N and water enrichment increased taxonomic divergence among replicate communities. However, only water addition, but not N enrichment, led to an increase in community phylogenetic convergence, which was associated with colonizing species in each water addition plot being more closely related to species in other replicate plots of the same treatment. These results suggest that although stochastic processes may cause communities to diverge in species composition, deterministic process could still drive communities to converge in phylogenetic community structure.

The third chapter describes an experiment aimed to identify and explore the importance of keystone communities in maintaining biodiversity and ecosystem functioning. Using laboratory protist microcosms as the model system, the effects of environmental uniqueness and location of local communities on their keystone-ness were studied. The removal of local communities with unique environmental conditions, which supported endemic species, reduced regional-scale diversity, therefore qualifying these communities as regional-scale keystone communities. These keystone communities also had large impacts on ecosystem functions, including biomass production and particulate organic matter decomposition. Moreover, keystone communities for biovolume production

were not keystone for organic matter decomposition, and vice versa. This study provides, to our knowledge, the first experimental evidence for keystone communities.

The last chapter explores the relative importance of deterministic and stochastic processes in shaping phyllosphere microbial communities in fragmented subtropical forests on the islands of Thousand-Island Lake in China. I collected shade leaves from tree species on ten islands and extracted microbial cells from leaf surfaces, and quantified bacterial and fungi community diversity and structure via high-throughput sequencing. The assembly of phyllosphere bacterial and fungal communities was highly stochastic. In addition to the dominant structuring role of stochastic processes, I also found evidence of deterministic processes shaping phyllosphere microbial communities, with host plant species identity and spatial distance among islands being the most important deterministic factor influencing the structure of phyllosphere bacterial and fungal communities, respectively.

CHAPTER 1.

**NITROGEN FERTILIZATION, NOT WATER ADDITION,
ALTERS PLANT PHYLOGENETIC COMMUNITY STRUCTURE
IN A SEMI-ARID STEPPE**

Abstract. Anthropogenic environmental changes, such as nitrogen (N) enrichment and alteration in precipitation regimes, significantly influence ecosystems world-wide. However, we know little about whether and how these changes alter the phylogenetic properties of ecological communities. Based on a 7-year field experiment in the temperate semi-arid steppe of Inner Mongolia, China, we investigated the influence of increased N and precipitation on plant phylogenetic structure and phylogenetic patterns of species colonization and extinction. Our study demonstrated that N and water addition influenced different aspects of plant community structure. Water addition increased plant species richness by preventing species extinction and facilitating species colonization, without altering community phylogenetic structure. In contrast, N addition did not alter species richness, but promoted the colonization of species distantly related to the residents, changing community phylogenetic structure from being neutral to overdispersion. We also found evidence for abundance-based extinction where rarer species were at greater risk of extinction, and functional trait-based species extinction where shorter statured plants and shallower rooted plants were at greater risk of extinction. Our study provides the first experimental evidence that plant phylogenetic community structure responds differently to different aspects of global changes. Importantly, the colonization of non-resident species,

rather than the extinction of resident species, contributed predominantly to changes in plant community phylogenetic structure in response to N amendment. Our findings highlight the importance of considering species phylogenetic relationships for a more complete understanding of anthropogenic influences on ecological communities.

Keywords: global change ecology, nitrogen fertilization, phylogenetic community structure, precipitation change, semi-arid steppe, species richness

1.1 Introduction

Anthropogenic environmental changes, such as nitrogen (N) deposition and changing precipitation, are affecting ecosystems world-wide (Vitousek et al., 1997). Both observational and experimental studies have shown that the addition of the two resources could result in considerable changes in community and ecosystem properties. Fertilization studies in N-limiting systems have found that increases in N availability tend to have positive effects on primary productivity, but negative effects on plant species richness (Bobbink et al., 1998; DeMalach et al., 2017a; Gough et al., 2000; Harpole et al., 2016; Suding et al., 2005). Water addition also often results in increases in primary production, whereas its effects on plant species richness are varied (DeMalach et al., 2017b; Knapp & Smith, 2001; Yang et al., 2011). Notably, much research on N and water addition effects has focused on ecosystem - level responses (e.g. primary production, ecosystem carbon exchange). Apart from species richness, other community-level responses to N and water manipulations have received relatively little attention (Yang et al., 2011). Here, we report,

to our knowledge, the first study that examined responses of plant phylogenetic community structure to changes in N and water availability.

Studying community phylogenetic structure could provide potential insight into how species and communities respond to environmental changes (Lavergne et al., 2010; Willis et al., 2008). For example, when functional traits are phylogenetically conserved, the coexistence of closely related species (phylogenetic clustering) may indicate the importance of local environment conditions selecting for species with similar traits, while the co-occurrence of distantly related species (phylogenetic overdispersion) may indicate that competitive exclusion among closely related species strongly influences community assembly (Cavender-Bares et al., 2009; Webb et al., 2002). According to this framework, phylogenetic clustering is expected to occur under resource deficiency, where only certain closely related species that are tolerant of low resource availability can persist (Graham et al., 2009). As a result, adding limiting resources, such as N and water, may be expected to alleviate environmental stress and reduce the level of phylogenetic clustering. However, recent work has provided a more nuanced view of a myriad of mechanisms influencing phylogenetic community structure (Cavender-Bares et al., 2009; Mayfield & Levine, 2010). For example, competition may lead to phylogenetic clustering, rather than phylogenetic overdispersion, when competitive outcome is largely determined by species competitive ability differences (Mayfield & Levine, 2010). Based on this revised framework, predicting changes in community phylogenetic structure in response to anthropogenic environmental changes may not be straightforward.

Anthropogenic environmental changes alter phylogenetic community structure through changing species extinction and/or colonization. Two contrasting hypotheses aim at explaining species extinction after environmental changes. First, the abundance-based hypothesis suggests that rare species are more likely to be lost after environmental changes than abundant species, given the greater probabilities for species with lower initial abundances to go locally extinct (Stevens & Carson, 1999; Suding et al., 2005). In situations where the abundance-based mechanisms are important, the random loss of rare species may not alter community functional/phylogenetic structure or may drive communities towards functional/phylogenetic overdispersion (Li et al., 2015); the extinction of a group of closely related rare species (i.e. conservatism in species abundance), however, could result in functional/phylogenetic clustering. Second, the functional trait-based hypothesis suggests that species losses are non-random, such that species carrying certain traits suffer greater extinction risk than other species. For example, species that are tolerant of low N levels, such as legumes, may be disadvantaged and experience increased extinction after N addition (Stevens et al., 2004; Xia & Wan, 2008). In situations where the functional trait - based mechanisms are important, the loss of a group of species that are phylogenetically and functionally distinct from the other resident species would drive the community towards functional and phylogenetic clustering (Li et al., 2015); loss of some, but not all of these species, however, may not necessarily cause significant changes in functional/phylogenetic structure. Likewise, species colonization could be either functional trait-based or random, causing different changes in phylogenetic and functional structure.

Here, we report on an experimental study conducted in a temperate steppe in northern China to explore the effects of N enrichment and increased precipitation on plant community phylogenetic structure. As an ecologically and economically important ecosystem stretching across much of the Eastern Europe and Central Asia, the temperate steppe is currently experiencing significant anthropogenic environmental changes including N deposition and increased precipitation (Chen et al., 2013; Niu et al., 2010; Xia et al., 2009). The potential impact of these changes on plant community phylogenetic structure and the underlying mechanisms, however, remain unexplored. Our experiment aimed to answer two specific questions. First, how does N and water amendment affect the phylogenetic structure of plant communities in the temperate steppe? Second, how do changes in species colonization and extinction, in response to N and water amendment, contribute to the changes in community phylogenetic structure?

1.2 Materials and methods

1.2.1 Study site and experimental design

This study was conducted at the Duolun Restoration Ecology Station of the Institute of Botany of Chinese Academy of Sciences, a long-term experimental study site of temperate steppe in the Inner Mongolia Autonomous Region of China (42°02'N, 116°17'E, 1324 m a.s.l.). The study area has a semi-arid continental monsoon climate with annual precipitation of 378 mm, most of which occurs from May to October. Mean annual temperature of this area is 2.1°C, with a mean monthly temperature of -17.5°C in January

and 18.9°C in July. Dominant plant species in the area are perennial herbs, including *Agropyron cristatum* and *Stipa krylovii* (Yang et al., 2012).

Our experiment employed a split-plot design with N addition as the primary factor and water addition as the secondary factor. We used four pairs of 45 m × 28 m plots, with the two plots in each pair assigned to the control and N addition treatments respectively. Within each main plot, two 15 m × 10 m subplots were established and assigned to the control and water addition treatments respectively. In the N addition plots, we added 10 g N m⁻² year⁻¹ in the form of urea in July 2005 and the form of NH₄NO₃ in July from 2006 to 2011. The N enrichment level applied was comparable to the total nitrogen deposition rate in northern China (about 8.33 g N m⁻² year⁻¹; He et al., 2007). In the water addition subplots, 15 mm of water was added weekly in July and August, resulting in a 30% increase in water supply each year to simulate the projected change in precipitation (Sun & Ding, 2010). In total, we used 16 plots of 15 m × 10 m, with four replicates for each of the four treatments (control, N addition, water addition and N plus water addition).

1.2.2 Vegetation sampling and plant trait collection

We surveyed our experimental plots using the point intercept method in August each year (from 2005 to 2011) when plant communities attained their peak biomass. In each 15 m × 10 m plot, we randomly selected a 1 m × 1 m permanent quadrat and placed a 1 m × 1 m frame with 100 10 cm × 10 cm grids above the canopy. We identified all species in each grid and estimated the coverage of each species based on their occurrence within the 100 grids.

We also collected data on plant height and rooting depth, two traits thought to influence plant responses to N and water addition in this region (Yang et al., 2011). The height of 17 common species was directly measured by randomly selecting ten individuals in the control plots; the height of uncommon species and the rooting depth of all species were extracted from the TRY database (Kattge et al., 2011). In total, we were able to collect data on plant height for 48 species and root depth for 25 species.

1.2.3 *Phylogenetic analyses*

We constructed a phylogenetic tree for the 58 species observed in the experimental area (Figure A. 1). Families and genera were constrained according to the phylogeny of vascular plants constructed by Zanne et al., (2014) and Qian and Jin (2016). In genera *Allium*, *Astragalus* and *Potentilla*, however, several species were absent in Zanne et al., and Qian & Jin's phylogeny. To improve the resolution of the phylogeny, we acquired the ITS1 and ITS2 sequences of these species from GenBank and constructed one phylogenetic tree for each genus. Specifically, we aligned the sequences from GenBank with Clustal X (version 2.0; Larkin et al., 2007), confirmed the alignment by observation, and selected the best evolution model (012340 + G + F for *Allium* and *Potentilla*; 011012 + F for *Astragalus*; note that the six-digit numbers represent substitution codes) with jModelTest (version 2.1.10; Darriba et al., 2012; Guindon & Gascuel, 2003), by using the Akaike information criterion for each genus. Using the closest relative to each genus as the outgroup, we constructed the phylogeny of each genus with the Bayesian method in MrBayes (version 3.1.2; Huelsenbeck & Ronquist, 2001) and the maximum likelihood method in the “phangorn” package in R (Schliep, 2011). The phylogenies constructed with the two

approaches were qualitatively similar. We, therefore, replaced the branches of these three genera in the phylogeny with the Bayesian trees.

We used abundance-weighted mean pairwise distance (MPD) as a measure of community phylogenetic diversity. MPD represents the average phylogenetic distance between two random individuals drawn from the focal community and is independent of species richness (Webb et al., 2008). We calculated abundance-weighted net relatedness index (NRI) as a measure of the phylogenetic structure of communities. NRI is defined as the difference in MPD between observed and randomly generated null communities, standardized by the standard deviation of phylogenetic distances in the null communities (Webb et al., 2008). The NRI was thus calculated as:

$$NRI_{sample} = -1 \times \frac{MPD_{sample} - MPD_{null}}{sd(MPD_{null})} \quad (1.1)$$

We created the null communities by shuffling species labels across the entire phylogeny, thus randomizing phylogenetic relationships among species, with 999 iterations. A positive NRI indicates phylogenetic clustering, whereas a negative NRI indicates phylogenetic overdispersion. We also calculated the mean nearest taxon distance (MNTD; Webb et al., 2008) and the nearest taxon index (NTI; Webb et al., 2008) as alternative measures of community phylogenetic diversity and structure respectively. Results based on these indices were similar to those based on MPD and NRI; we thus only present the results based on MPD and NRI here.

We classified species as locally extinct from a plot if they were present in 2005 but absent in 2011, and defined new colonists as species that were absent in 2005 but present in 2011. To examine the contribution of new colonists to community phylogenetic structure, we calculated β NTI to quantify the phylogenetic similarities between new colonists and residents of each plot during the 7-year period. β NTI is the standardized value of the mean nearest taxon phylogenetic distance between the newly emerged species and resident species in our study (β MNTD; Webb et al., 2008), and is highly correlated with other phylogenetic community similarity indices such as Phylosor and UniFrac (Swenson, 2011). β NTI was calculated as:

$$\beta NTI = -1 \times \frac{\beta MNTD_{observed} - \beta MNTD_{null}}{sd(\beta MNTD_{null})} \quad (1.2)$$

We used the same method for calculating NTI to construct null models for calculating β NTI. A positive β NTI indicates that new colonists are more closely related to the residents than by chance, whereas a negative β NTI indicates the opposite. To examine the contribution of locally extinct species to community phylogenetic structure, we also calculated the β MNTD and β NTI between locally extinct species and resident species for each plot. We examined the phylogenetic signal of species colonization and extinction using the D statistic, which quantifies the strength of phylogenetic signal for binary traits (Fritz & Purvis, 2010). We calculated the D statistic at the treatment level where a species was categorized as an extinct species when it went extinct in any of the four replicate plots and was categorized as a colonist species when it colonized any of the four replicate plots. We calculated the D statistic at the treatment level for two reasons. First, the number of

extinct/colonized species in some plots was relatively low (ranging from one to eight for colonized species and three to 12 for extinct species), making it difficult to detect any phylogenetic pattern. Examining the data at the treatment level helped improve the power of statistical tests. Second, several species went extinct in one plot but colonized another plot within the same treatment, presumably the result of stochasticity associated with extinction and colonization events. We categorized these species as neither extinct nor colonist at the treatment level, reducing the influence of stochasticity on our results. D value of 1 indicates that the distribution of a binary trait is random on the phylogeny; D values that are greater than 1 indicate that the trait is more overdispersed than expected by chance; D values that are smaller than 1 indicate that the trait is more phylogenetically clumped than expected by chance. D statistic was calculated based on 1,000 permutations. In addition, we also calculated the K statistic (Blomberg et al., 2003) as another measure of phylogenetic signal of species extinction/colonization, with species extinction/colonization probability calculated as the proportion of replicates in which a species went extinct/colonized within each treatment.

The phylogenetic diversity and structure of communities were calculated using the *picante* package (Kembel et al., 2010) in R (R Development Core Team, 2014). The D statistic was calculated using the “*phylo.d*” function of the *caper* package (Orme, 2013) in R.

1.2.4 Statistical analysis

We used linear mixed-effect models (LMM) to test for the effects of N and water addition on MPD and NRI over time, in which we treated N treatment, water treatment, their interaction, and year as fixed factors, and the four blocks and two plots nested within each block as random factors. Generalized linear mixed-effect models (GLMMs) with Poisson error distribution were used to test the effects of the same variables on species richness. We examined the variance explained by the fixed factors in the LMM and GLMM models using marginal R^2 (Nakagawa & Schielzeth, 2013). To assess the importance of species' functional traits on extinction and colonization, we ran logistic regressions of species extinction and colonization as functions of species' trait values (height and root depth). To assess the importance of species initial coverage on extinction, we ran logistic regressions of species extinction as a function of the initial coverage of species present at the beginning of our experiment (i.e. 2005). Species that did not colonize or go extinct in any plot within a treatment were assigned a value of 0. Otherwise, species were assigned a value of 1. The assignments of values were done separately for colonization and extinction. We also ran logistic regressions on the likelihood of species extinction and colonization by defining species extinction and colonization probabilities as proportional variables (i.e. the proportion of replicates in which a species went extinct/colonized within each treatment). All statistical analyses were conducted using R (R Core Team, 2014). LMMs and GLMMs were performed with the lme4 R package (Bates et al., 2015), and marginal R^2 was obtained with the *r.squaredGLMM* function in the MuMIn R package (Bartoń, 2015).

1.3 Results

1.3.1 *Species richness, phylogenetic diversity and community phylogenetic structure*

Plant species richness declined as the experiment progressed in all but the water addition treatments (Figure 1.1; Table 1.1). Species richness in N addition plots declined precipitately initially, but the effect of N addition dissipated after 2009, resulting in similar number of species between the N addition and control plots at the end of the experiment (Figure 1.1a). The mixed - effect model revealed that water addition significantly increased species richness, whereas N addition did not influence species richness (Table 1.1). The effect of water addition on species richness increased over time, resulting in a significant water \times year term in the mixed - effect model (Table 1.1). In contrast, although water addition did not influence MPD or NRI, nitrogen addition significantly increased MPD and decreased NRI (Table 1.1b,c; Table 1.1). NRI was not significantly different from zero at the beginning of the experiment in all treatments (One sample t test, $p = 0.803, 0.968, 0.954, 0.753$ for the control, N, Water and N + Water addition plots respectively), and was significantly smaller than zero at the end of the experiment only in the N addition treatment (One sample t test, $p = 0.220, 0.023, 0.158, 0.124$ for the control, N, Water and N + Water addition plots respectively), indicating that N addition reduced average phylogenetic relatedness among species and changed the phylogenetic structure from neutral to overdispersion.

Table 1.1 – Results of generalized linear mixed-effect model (GLMM) and linear mixed-effect model (LMM) on the effects of N addition, water addition, their interaction, and time on species richness, mean pairwise distance (MPD), and net relatedness index (NRI). Significant p values are shown in bold (p<0.05).

	Num. DF	Den. DF	Species richness		MPD		NRI	
			Z	p	F	p	F	p
N	1	98	0.211	0.833	5.454	0.022	17.033	<0.001
Water	1	98	-2.004	0.045	1.133	0.290	0.008	0.929
Year	1	98	-2.638	0.008	14.441	<0.001	0.000	0.996
N*W	1	98	0.715	0.475	0.006	0.938	0.265	0.608
N*Y	1	98	-0.210	0.834	5.468	0.021	2.055	0.155
W*Y	1	98	2.007	0.045	1.135	0.289	0.133	0.716
N*W*Y	1	98	-0.717	0.473	0.006	0.938	0.368	0.545
Marginal R ²			0.230		0.216		0.151	

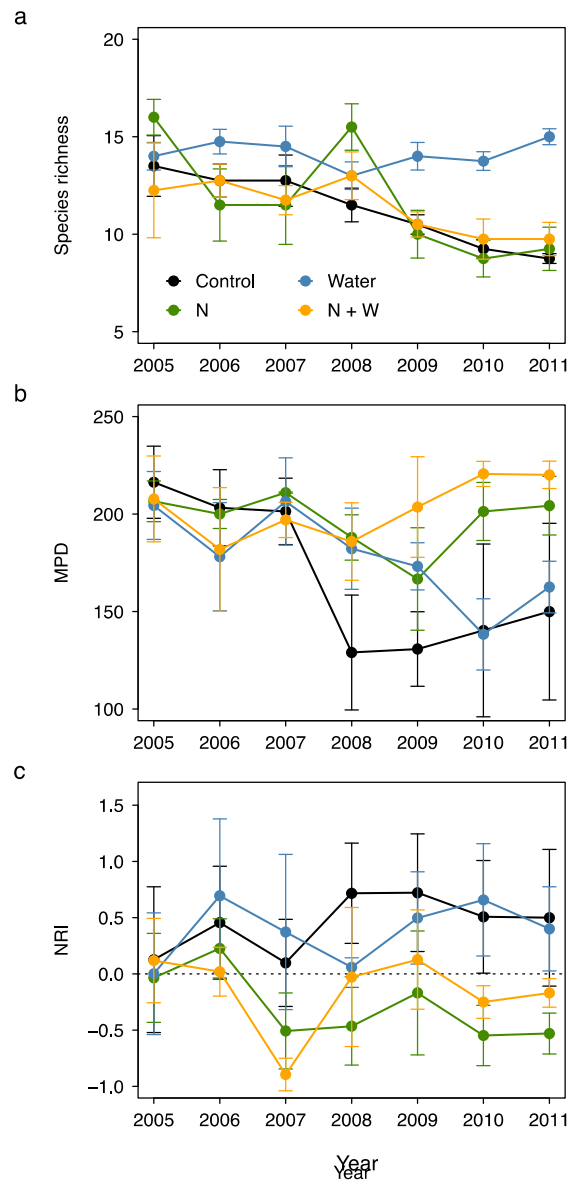


Figure 1.1 – The effects of N and water addition on species richness, mean pairwise distance (MPD) and net relatedness index (NRI) over time. Error bars represent standard errors.

1.3.2 *Species colonization and extinction*

During our experimental period, an average of 7.25 (SE = 1.55) species went extinct and 2.5 (SE = 0.29) species colonized in the control plots. By comparison, an average of 8.75 (SE = 1.18) species went extinct and two (SE = 0.58) species colonized in the N addition plots. Most of the extinct species in the control and N addition plots were grasses from the family Poaceae and forbs from the genera *Allium* and *Potentilla*. In the water addition plots, however, only an average of 4.75 (SE = 0.85) species went extinct, with most being extinct also in the control plots (Figure A. 1). An average of 5.75 (SE = 1.10) species colonized the water addition plots, and most of these species did not colonize the control plots (Figure A. 1). In plots with both N and water addition, an average of six (SE = 1.58) species went extinct and 3.5 (SE = 1.19) species colonized.

Species extinction showed significant phylogenetic clustering in the control ($D = 0.75$, $p_{\text{random}} = 0.023$) and water addition ($D = 0.68$, $p_{\text{random}} = 0.017$) treatments (Figure 1.2a). In contrast, the phylogenetic pattern of species extinction did not deviate from random expectations in the N addition or N plus water addition treatments. Species colonization showed significant phylogenetic clustering ($D = 0.73$, $p_{\text{random}} = 0.045$) only in the N addition treatment, whereas the phylogenetic pattern of species colonization in the other treatments did not differ from random expectations (Figure 1.2a). Phylogenetic signal measured by the K statistic was also significant for species extinction in the controls ($K = 0.124$, $p = 0.04$) and for species colonization in the N addition ($K = 0.248$, $p = 0.01$) treatments (Table A. 1). βNTI between locally extinct species and resident species did not differ from zero in all treatments, indicating phylogenetic randomness of species extinction

(Figure 1.2b). β NTI between colonists and residents in the control and water addition also did not differ from zero (Figure 1.2b). However, we found significant negative colonizer-resident β NTI in the N addition (Figure 1.2b; $p = 0.044$) and N + Water addition (Figure 1.2b; $p = 0.048$) plots, indicating that colonizing species in these treatments were more distantly related to the residents than expected by chance (Figure 1.2b).

The initial coverage of species was a significant predictor of species extinction in all treatments, where species with lower initial coverage tended to have a greater probability of extinction (Figure 1.3a-d). In the control plots, species extinction was also influenced by plant height where species with shorter stature suffered greater risk of extinction (Figure 1.3e). In water addition plots, rooting depth also influenced the likelihood of species extinction, with shallow - rooted species being more likely to be lost (Figure 1.3k). However, neither height nor root depth was a significant predictor of species colonization. The results were similar when species extinction and colonization probabilities were defined as proportional variables (Figure A. 2).

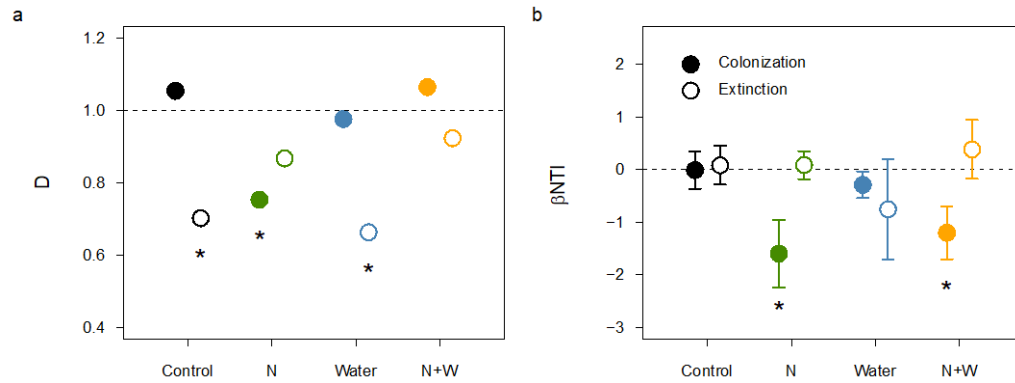


Figure 1.2 – The phylogenetic signal of species colonization and local extinction, and the phylogenetic dissimilarity of colonist and locally extinct species to the resident species. (a) The phylogenetic signal was quantified by the D statistic. D smaller than 1 indicates the trait is more clumped than expected by chance, while D greater than 1 suggests the opposite. (b) The phylogenetic dissimilarity was calculated as β NTI by comparing the observed values to the null models. Negative values indicate that the colonists or extinct species are more closely related to the resident species than expected by chance, while positive values suggest the opposite. * in (a) denotes values that are significantly different from random expectation based on simulation tests ($p < 0.05$), and * in (b) denotes values that are significantly different from zero based on one-sample t test ($p < 0.05$). Error bars represent standard errors.

1.4 Discussion

Anthropogenic environmental changes, such as increased N deposition and precipitation, are altering Earth's ecosystems. While many studies have explored the ecological consequences of these changes, our study differs from these studies by adopting the phylogenetic perspective in assessing their impacts, and by linking both species colonization and extinction to the observed impacts. By so doing, our study produced two main findings that have not been reported previously. First, N and water amendment influenced different aspects of plant community structure. Specifically, water, but not N, addition increased plant species richness, whereas N, but not water, addition altered plant community phylogenetic structure, driving communities towards phylogenetic overdispersion. Second, the observed change in community phylogenetic structure towards overdispersion under N addition was associated with the colonization of species that were distantly related to the resident species, rather than the extinction of resident species.

1.4.1 Effects of N and water addition on species richness

We found no significant effect of N addition on plant species richness, which appears at odds with the widely reported adverse effect of N enrichment on plant species richness in grasslands (DeMalach et al., 2017a; Gough et al., 2000; Harpole et al., 2007; Harpole et al., 2016; Yang et al., 2011). Addition of N has the potential to reduce plant species richness, as suggested by theories such as the light competition hypothesis (Dickson & Foster, 2008; Dybzinski & Tilman, 2007; Hautier et al., 2009) and the niche

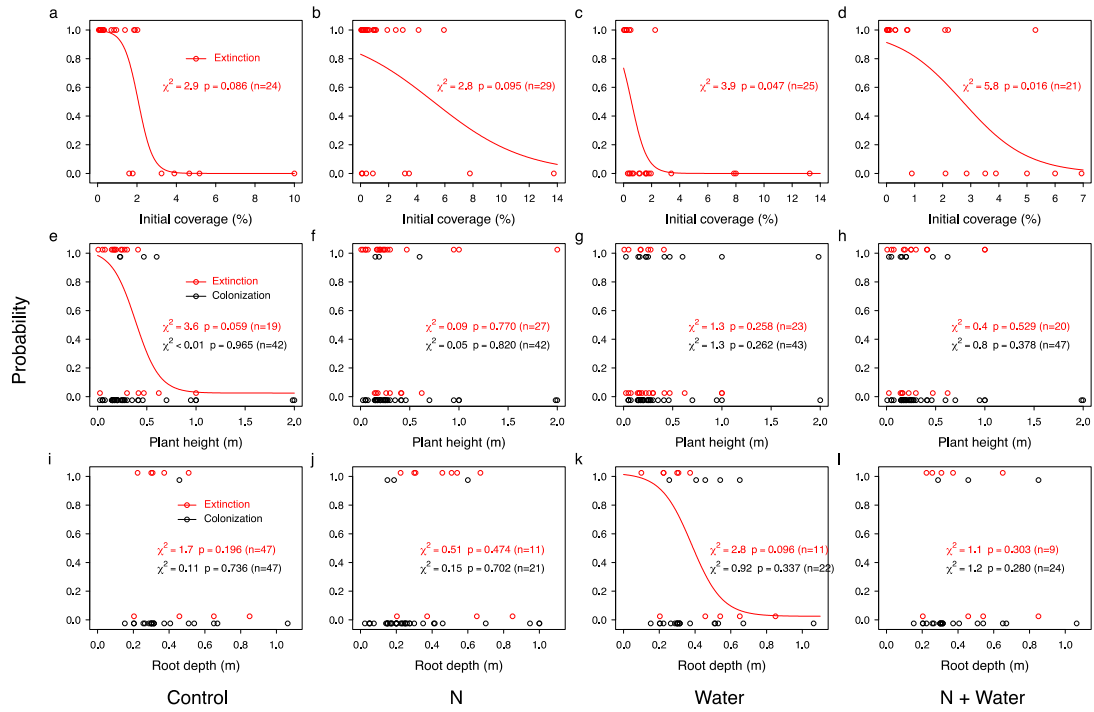


Figure 1.3 – Species colonization and local extinction as functions of initial abundance, plant height and root depth in each treatment. Species that did not colonize or go extinct in any plot within a treatment was assigned a value of 0. Otherwise, species were assigned a value of 1. Significant logistic regression lines ($p < 0.1$) are shown.

dimension hypothesis (Harpole & Tilman, 2007; Harpole et al., 2016; Tilman, 1982), all under the assumption with N being the limiting nutrient. However, our work pointed to water, rather than N, as the primary limiting resource in our study system, given that there was a significant increase in plant cover after water addition, but not after N addition (Figure A. 3). Li et al., (2011) also reported similar response of plant biomass to water and N manipulations at the same experimental site. Likewise, Diekmann et al., (2014) reported the lack of response of plant species richness to N deposition in calcareous grasslands, where phosphorus and water are more limiting than N. These results are consistent with the idea that plant species richness would be reduced to the largest extent by N addition only when N is the primary limiting resource and when there is a large increase in productivity after N addition (Clark et al., 2007).

In contrast to the lack of N addition effect on species richness, increased water availability led to an increase in plant species richness (Table 1.1; Figure 1.1). This positive effect of water addition on plant diversity arose from reduced species extinction and increased species colonization under increased water availability, which promotes seedling survival and plant growth in dry seasons (Yang et al., 2011; Zavaleta et al., 2003). This result is therefore inconsistent with the light competition hypothesis, which suggests that increased plant biomass, as the result of resource enrichment, would strengthen competition for light and increase species extinction (Dickson & Foster, 2008; Dybzinski & Tilman, 2007; Hautier et al., 2009). It is also inconsistent with the niche dimension hypothesis, which suggests that the addition of a primary limiting resource would reduce

the dimensionality of resource trade-offs for plants, and in turn, the number of coexisting plant species (Harpole & Tilman, 2007; Harpole et al., 2016; Tilman, 1982).

It is notable that while some studies have reported no or negative water addition effect on plant diversity (Goldberg & Miller, 1990; Suttle et al., 2007), a number of other studies, including ours, have documented a positive effect of water addition on plant diversity (Cornwell & Grubb, 2003; Xu et al., 2012; Yang et al., 2011; Zavaleta et al., 2003). This discrepancy may be potentially explained by the timing of water addition. In their phenology hypothesis, Goldberg & Miller (1990) proposed that resource enrichment applied in the earlier growing season is more likely to accelerate canopy closure and cause competitive exclusion. Consistent with this hypothesis, Suttle et al., (2007) found that spring water addition in a Californian grassland promoted the production of annual grasses, resulting in the elimination of forbs and reduction in plant species richness, whereas water addition in the late growing season did not alter plant species richness. In our experiment, water was applied in the middle of the growing season (July and August); future experiments should investigate whether earlier water addition reduces plant diversity in the semi-arid steppe we studied, as suggested by the phenology hypothesis. Note that adding N and water together removed the positive effect of water addition on species richness in our experiment, which is consistent with the finding of a recent meta-analysis (DeMalach et al., 2017b). One possible explanation for this result is that increased photosynthetic rate after N addition led to greater transpiration rate and more rapid depletion of water from the rooting zone and therefore reduced soil moisture (Harpole et al., 2007; Zavaleta et al., 2003), counteracting the effect of direct water addition.

1.4.2 Effects of N and water addition on community phylogenetic structure

Our study found increased phylogenetic overdispersion after N addition, which is in line with our hypothesis that the addition of limiting resources would reduce the level of phylogenetic clustering by alleviating environmental stress. The initial coverage of species was significantly associated with their extinction probability in all treatments, suggesting that abundance was consistently an important predictor of species loss (Figure 1.3a–d). However, this extinction pattern cannot explain the transition of community phylogenetic structure from neutral to overdispersion after N addition, because of phylogenetic randomness of the extinct species in this treatment (Figure 1.2a,b). In contrast, species colonization was functional trait based in N addition plots because the colonists showed significant phylogenetic signal and were distantly related to resident species (Figure 1.2a,b). The change in community phylogenetic structure from neutral to overdispersion was therefore driven by the colonization of species that were distantly related to the resident species, rather than the extinction of resident species. This result is akin to the finding of a recent study that the transition of plant communities towards phylogenetic overdispersion over long - term succession was mainly driven by the colonization of species that were distantly related to the residents, rather than the exclusion of closely related residents (Li et al., 2015). Together, these results suggest that more attention should be paid to the colonization of species, in addition to their extinction, in explaining temporal changes in community phylogenetic structure patterns. Finally, we note that N addition in our experiment differs from natural forms of N deposition, which are mainly atmospheric and

contain different substances than those used in our experiment. It would be worthwhile to explore whether our findings would hold for other forms of N deposition.

Water addition did not significantly affect community phylogenetic structure. However, we found functional trait-based extinction in the control and water addition plots. Species extinction in control and water addition plots exhibited significant phylogenetic signal and showed a clumped distribution, indicating that extinct species were more closely related to each other than expected by chance alone in the two treatments. Correspondingly, the probability of local extinction was strongly associated with plant height in the control plots and with rooting depth in the water addition plots. Plant height is a key functional trait related to light competition as short - stature plants suffer more from reduced light availability. The greater extinction probability of short-stature species in the control plots is consistent with findings of other observational (e.g. Leach & Givnish, 1996) and experimental (e.g. Huang et al., 2013) studies, supporting the hypothesis that low-stature species tend to be suppressed by high-stature species when competing for light (Dickson & Foster, 2008; Dybzinski & Tilman, 2007; Hautier et al., 2009). Rooting depth influences plants' ability in water uptake and is associated with below-ground competition among species (Cornelissen et al., 2003). Shallow-rooted plants had a greater risk of extinction after water addition, presumably because water penetrated into deep soil after pulsed water addition favours deep - rooted plants over shallow - rooted plants (Chesson et al., 2004). Probably for this reason, plant communities' transition from domination by shallow-rooted grass to domination by deep-rooted woody species after increased precipitation in arid and semi-arid areas (Archer et al., 2017; Good & Caylor, 2011). Note that neither trait was a

significant predictor of species colonization, likely because colonization was more affected by regional processes such as species' dispersal distance and their regional population size (Ricklefs, 2004), which are themselves influenced by traits not considered in our study.

Our study demonstrated that the N and water addition influenced different aspects of plant community structure. Water addition increased species richness by reducing the extinction of resident species and increasing the number of new colonists, without causing changes in community phylogenetic structure. N addition altered phylogenetic community structure by promoting the colonization of species that are distantly related to the local species, without changing species richness. Importantly, the colonization of non-resident species, rather than the extinction of resident species, contributed predominantly to changes in plant community phylogenetic structure after N fertilization. Our study illustrates the value of considering phylogenetic information for a more comprehensive understanding of the responses of ecological communities to anthropogenic environmental changes. The generality of our findings, nevertheless, remains to be assessed by future studies. We hope our work will stimulate the rapid emergence of these studies, given a number of field experiments similar to ours have already been conducted (reviewed by DeMalach et al., 2017b).

CHAPTER 2.

RESOURCE ADDITION DRIVES TAXONOMIC DIVERGENCE

AND PHYLOGENETIC CONVERGENCE OF

PLANT COMMUNITIES

Abstract. Anthropogenic environmental changes are known to affect the Earth's ecosystems. However, how these changes influence assembly trajectories of the impacted communities remains a largely open question. In this study, we investigated the effect of elevated nitrogen (N) deposition and increased precipitation on plant taxonomic and phylogenetic β -diversity in a 9-year field experiment in the temperate semi-arid steppe of Inner Mongolia, China. We found that both N and water addition significantly increased taxonomic β -diversity, whereas N, not water, addition significantly increased phylogenetic β -diversity. After the differences in local species diversity were controlled using null models, the standard effect size of taxonomic β -diversity still increased with both N and water addition, whereas water, not N, addition, significantly reduced the standard effect size of phylogenetic β -diversity. The increased phylogenetic convergence observed in the water addition treatment was associated with colonizing species in each water addition plot being more closely related to species in other replicate plots of the same treatment. Species colonization in this treatment was found to be trait-based, with leaf nitrogen concentration being the key functional trait. Our analyses demonstrate that anthropogenic environmental changes may affect the assembly trajectories of plant communities at both taxonomic and phylogenetic scales. Our results also suggest that while stochastic processes may cause

communities to diverge in species composition, deterministic process could still drive communities to converge in phylogenetic community structure.

Keywords: community assembly, global change ecology, nitrogen fertilization, phylogenetic β -diversity, precipitation change, semi-arid steppe, taxonomic β -diversity

2.1 Introduction

Understanding mechanisms driving compositional variation across ecological communities, frequently referred to as β -diversity, is one of the major goals of community ecology (Anderson et al., 2011; Chase & Myers, 2011; Whittaker, 1960). Across large spatial scales, evolutionary and biogeographic processes such as in situ diversification may constitute an important source of β -diversity (Graham & Fine, 2008; Ricklefs, 2006, 2008). Ecological theories that explain β -diversity among communities, which generally ignore evolutionary processes, fall into two broad categories. The niche theory suggests that β -diversity arises largely from deterministic processes, driven by ecological selection favouring different species across localities characterized by different environmental conditions (Chase & Leibold, 2003). In contrast, the neutral theory suggests that β -diversity could simply arise from ecological drift, driven by stochastic processes such as chance colonization and random demographic events (Bell, 2001; Hubbell, 2001). Ecological communities are known to be subject to the influence of both niche - based ecological selection, which would cause communities sharing similar environmental conditions to be structurally similar (i.e. low β -diversity), and stochasticity-based ecological drift, which

could cause substantial structural dissimilarity among communities (i.e. high β -diversity) even in similar environments (Adler et al., 2007; Gravel et al., 2006; Leibold & McPeck, 2006). As anthropogenic environmental changes, such as increased nitrogen (N) deposition and changing precipitation, continue to affect ecosystems world - wide (Vitousek et al., 1997), it is essential to understand how these changes affect the relative importance of the two contrasting processes in shaping community assembly, and, consequently, β -diversity among the assembled communities.

Anthropogenic environmental changes in the form of resource amendment (e.g. increased N deposition and elevated precipitation) may have the potential to impact the trajectory of community assembly, and thus β -diversity in opposite directions. For example in environments where limited resource supply presents an important environmental filter that excludes many species whose resource requirements are not met, increased resource input may relieve environmental harshness and allow a greater number of species to successfully colonize the habitat; the resulting larger species pool could then more readily give rise to alternative community states (Chase, 2003; Fukami, 2004; Jiang et al., 2011; Law & Morton, 1993; Levine et al., 2017; Saavedra et al., 2017), resulting in increased β -diversity. The increased environmental productivity under resource enrichment may further promote the presence of alternative community states (Chase, 2010; Ejrnæs et al., 2006; Isbell et al., 2013). On the other hand, increased resource input may favour species with certain traits but make the condition less favourable for other species (e.g. Dickson et al., 2014), which would lead to increased dominance of the same species assemblages across communities, resulting in reduced β -diversity. In addition, resource enrichment may

often cause the reduced availability of other resources and increased intensity of competition for these resources (e.g. light for plants, Hautier et al., 2009), accelerating deterministic competitive exclusion.

Notably, existing studies on the response of β -diversity to environmental changes have focused on taxonomic β -diversity that captures turnover in species composition among sites (Chase, 2007, 2010; Myers et al., 2015; Zhang et al., 2011). It is less clear how phylogenetic β -diversity, which accounts for evolutionary relationships among species, responds to environmental changes [but see Guo et al., (2018) for a study of climate warming on soil microbial communities]. Studying phylogenetic β -diversity, however, could provide novel insight into how communities respond to environmental changes beyond those obtained via studying taxonomic β -diversity alone (Gerhold et al., 2015; Graham & Fine, 2008; Hardy et al., 2012). For instance, the study of both taxonomic and phylogenetic β -diversity could allow the exploration of the idea that the degree of determinism in community assembly may depend on the level of ecological organization examined (Diamond, 1975; Fox, 1987; Fukami et al., 2005). A group of phylogenetically closely related species may exhibit largely similar responses to environmental changes, by virtue of their similar traits, making the group-level response more deterministic. However, changes in individual species within the group may be less deterministic, as the result of ecological drift influencing populations of closely related species. We thus hypothesize that taxonomic and phylogenetic β -diversity may not necessarily show similar responses to environmental changes.

Changes in species taxonomic and phylogenetic β -diversity may be better understood by looking into species extinction and colonization patterns. Species loss may depend on their traits and evolutionary history, such that species of certain clades may suffer greater extinction risk than species of other clades (Purvis et al., 2000). For example, legumes, which can well tolerate low soil N concentrations, may experience elevated extinction under elevated N levels (Stevens et al., 2004; Xia & Wan, 2008). The deterministic loss of these clades across communities, in response to environmental changes, would promote community convergence, resulting in reduced taxonomic and phylogenetic β -diversity (Figure 2.1a). Phylogenetic β -diversity, however, may not necessarily decline as rapidly as taxonomic β -diversity if only some, not all species belonging to the same clades face extinction. Extinction, however, is far from deterministic (Lande, 1993; Lande et al., 2003). The random loss of species, especially those with small population sizes (Matthies et al., 2004; Suding et al., 2005), in different locations may drive divergence of species composition among communities, resulting in increased taxonomic and phylogenetic β -diversity (Figure 2.1b,c). Likewise, colonization could be either stochastic or deterministic, causing corresponding changes in taxonomic and phylogenetic β -diversity (Figure 2.1d,f,g). Colonization-induced changes in phylogenetic β -diversity also may not necessarily parallel those in taxonomic β -diversity. For example, the colonization of a group of species in which members are closely related would drive the communities towards phylogenetic convergence, but may not necessarily cause taxonomic convergence if other factors, such as dispersal limitation, prevent the same species from colonizing all localities. Under this circumstance, we would expect increased taxonomic

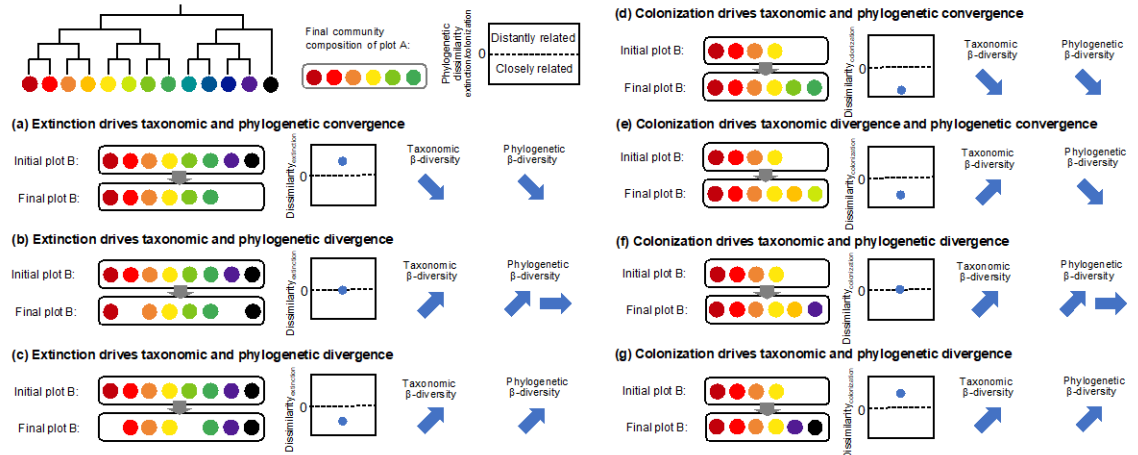


Figure 2.1 – A conceptual diagram of the potential effects of species colonization and extinction on community taxonomic and phylogenetic beta diversity. Plot A and B are two replicate plots under the same experimental treatments. For illustration purpose, here we only relate colonization/extinction in plot B (focal plot) to communities in plot A (reference plot). The overall effects of species colonization and extinction on community taxonomic and phylogenetic beta diversity could be assessed by averaging all possible pairwise combinations of replicate plots that are subject to the same treatments. Phylogenetic dissimilarity_{colonization/extinction} is the standardized effect size of phylogenetic β -diversity between colonized/extinct species in plot B and final community composition of plot A. (a) Extinction eliminates species in plot B that are distantly related to final species composition in plot A, leading to decreased taxonomic and phylogenetic beta diversity between the two plots. (b) Random extinction of species in plot B leads to increased taxonomic beta diversity and increased or unchanged phylogenetic beta diversity between the two plots. (c) Extinction eliminates species in plot B that are closely related to final species composition in plot A, leading to increased taxonomic and phylogenetic beta diversity between the two plots. (d) Colonization of species into plot B leads to similar final species composition in the two plots, leading to decreased taxonomic and phylogenetic beta diversity between the two plots. (e) Colonization of species into plot B that are not present in plot A but closely related to final species composition in plot A leads to increased taxonomic but decreased phylogenetic beta diversity between the two plots. (f) Random colonization of species into plot B leads to increased taxonomic beta diversity and increased or unchanged phylogenetic beta diversity between the two plots. (g) Colonization of species that are distantly related to final species composition in plot A into plot B leads to increased taxonomic and phylogenetic beta diversity between the two plots.

β -diversity but decreased phylogenetic β -diversity (Figure 2.1e).

Here, we report on a field experiment, conducted in a temperate steppe in northern China, to investigate the effects of N and precipitation amendment on taxonomic and phylogenetic β -diversity of plant communities. The temperate steppe in this area is currently experiencing significant anthropogenic environmental changes, including increased N deposition and precipitation (Chen et al., 2013; Niu et al., 2010; Xia et al., 2009), necessitating a thorough understanding of their ecological consequences. Previous work at the study site has documented changes in a number of community and ecosystem properties, including functional group composition (Yang et al., 2011), ecosystem productivity, respiration and net C exchange (Niu et al., 2009, 2010), community stability (Yang et al., 2012) and plant phylogenetic community structure (Yang et al., 2018), in response to experimental manipulations of N and precipitation. However, the question of how these environmental changes affect the trajectory of community assembly remains unanswered. We showed that both N and water addition increased the standard effective size of taxonomic β -diversity, whereas water, not N, addition, reduced the standard effective size of phylogenetic β -diversity, suggesting that anthropogenic environmental changes differentially affected plant community assembly trajectories at taxonomic and phylogenetic scales.

2.2 Materials and methods

2.2.1 Study site, experimental design and vegetation sampling

The experiment was conducted in a natural grassland at the Duolun Restoration Ecology Station of the Institute of Botany, Chinese Academy of Sciences, located in a temperate steppe (42°02' N, 116°17' E) in Inner Mongolia, China. The study area has a semi - arid continental monsoon climate with annual precipitation of 378 mm and mean annual temperature of 2.1°C. Dominant plant species (in terms of cover) in this area are perennial grasses and forbs, including *Stipa krylovii*, *Artemisia frigida*, *Potentilla acaulis*, *Cleistogenes squarrosa*, *Allium bidentatum* and *Agropyron cristatum*. Our study site was heavily grazed by livestock prior to 2001; it has been fenced since 2001 to exclude large herbivores.

The experiment used a split-plot design with N addition being the primary factor and water addition being the secondary factor. Four pairs of 45 m × 28 m plots were established in 2005, with two plots in each pair assigned to the control and N addition treatments respectively. Within each plot, we set up two 15 m × 10 m subplots assigned to the control and water addition treatments respectively. N enrichment was accomplished by adding 10g N m⁻² year⁻¹ in July 2005 in the form of urea and in July from 2006 to 2013 in the form of NH₄NO₃. The rate of natural N deposition in the study area is approximately 1.47g N m⁻²year⁻¹ (Zhang et al., 2017), and the amount of N addition applied is comparable to the rate of atmospheric nitrogen deposition in the North China Plain (about 8.33 g N m⁻²year⁻¹; He et al., 2007), where agricultural activities and fossil fuel consumption are

more concentrated. Water addition was conducted by adding 15 mm of water weekly in July and August, resulting in an approximately 30% increase in water supply each year. More detailed information on the study area and experimental design can be found in Yang et al., (2012).

We surveyed the experimental plots in August each year from 2005 to 2013. In each plot, we placed a 1 m \times 1 m frame with 100 10 m \times 10 cm grids into a randomly selected 1 m \times 1 m quadrat. All species in each grid were identified and their coverage were estimated based on their occurrence within the 100 grids. We also collected data on key plant functional traits, including plant height, rooting depth, leaf N concentration and specific leaf area (SLA). Unlike our previous work (Yang et al., 2018), which extracted most trait data from the TRY database (Kattge et al., 2011), here we measured most data in situ. Plant height was measured as the maximum height of each species in the experimental plots at the beginning of the experiment. Plant samples for the measurement of other traits were collected from a nearby grassland outside the experimental plots. Rooting depth, leaf N concentration and SLA of 26 common species were measured according to Cornelissen et al., (2003). For species for which trait data were not directly measured, we extracted data on rooting depth, leaf N concentration and SLA from the TRY database (Figure B. 1; Kattge et al., 2011).

2.2.2 *Phylogenetic tree*

We constructed a phylogenetic tree for the species observed in the experimental area (Figure B. 1). First, we built a genus-level phylogenetic tree based on the phylogeny

of vascular plants generated by Zanne et al., (2014) and Qian & Jin (2016). However, species in the genera *Allium*, *Astragalus* and *Potentilla* were absent from Zanne et al., and Qian & Jin's phylogeny. We thus extracted the ITS1 and ITS2 sequences of species belonging to these genera from GenBank and constructed a phylogenetic tree for each genus. We aligned the sequences from GenBank with Clustal X (version 2.0; Larkin et al., 2007), confirmed the alignment by observation, and selected the best evolution model with jModelTest (version 2.1.10; Guindon & Gascuel, 2003; Darriba et al., 2012; 012,340 + G+F for *Allium* and *Potentilla*; 011,012 + F for *Astragalus*). The phylogeny of each genus was constructed with the Bayesian method in MrBayes (version 3.1.2; Huelsenbeck & Ronquist, 2001), using the closest relative to each genus as the outgroup.

2.2.3 *Species and phylogenetic β -diversities and their standardized effect sizes*

To assess treatment effects on community convergence/divergence, we calculated dissimilarities in species composition (i.e. taxonomic β -diversity) and phylogenetic structure (i.e. phylogenetic β -diversity) between replicated plots within the same treatment. Taxonomic β -diversity was calculated using the abundance - weighted Bray - Curtis dissimilarity index (Bray & Curtis, 1957). The Bray-Curtis index is robust to sampling errors (Schroeder & Jenkins, 2018), and is widely used to quantify taxonomic β -diversity among communities. The value of Bray-Curtis dissimilarity approaches 0 when species composition is identical, and approaches 1 when species composition is completely different. Phylogenetic β -diversity was quantified using the abundance-weighted pairwise dissimilarity index D_{pw} (Swenson, 2011; Webb, Ackerly, & Kembel, 2008). D_{pw} is suitable

for detecting phylogenetically basal turnover between communities and converges to the Bray–Curtis dissimilarity index in the case of a star phylogeny (Swenson, 2011). It is calculated as:

$$D_{pw} = \frac{\sum_{i=1}^{n_{k_1}} f_i \overline{\delta_{ik_2}} + \sum_{j=1}^{n_{k_2}} f_j \overline{\delta_{jk_1}}}{2} \quad (2.1)$$

where k_1 and k_2 are two communities, f_i is the relative cover of species i in community k_1 , f_j is the relative cover of species j in community k_2 , $\overline{\delta_{ik_2}}$ is the mean pairwise phylogenetic distance between species i in community k_1 and all species in community k_2 excluding conspecific species, and $\overline{\delta_{jk_1}}$ is the mean pairwise phylogenetic distance between species j in community k_2 and all species in community k_1 excluding conspecific species. Larger values of D_{pw} indicate greater phylogenetic distance between the compared communities.

In addition to niche-based and stochasticity-based ecological processes, the observed patterns of taxonomic and phylogenetic β -diversity may also be affected by both local community diversity (α -diversity) and the size of the regional species pool (γ -diversity). In particular, when the regional species pool remains unchanged, any factor that changes α -diversity could potentially alter β -diversity owing simply to random sampling effects (Anderson et al., 2011; Chase et al., 2011; Chase & Myers, 2011; Myers et al., 2013). Therefore, we performed null model analyses to disentangle the variation in β -diversity from variation in α -diversity. The null model analyses determined if the observed patterns in taxonomic and phylogenetic β -diversity deviated from the expectations of random assembly, after accounting for changes in α -diversity. A null distribution of

taxonomic β -diversity was generated by randomly sampling individuals from the regional species pool 999 times, while persevering the total plant cover in each plot and the relative cover of each species in the species pool (Kraft et al., 2011). Null distributions of phylogenetic β -diversity were generated by randomizing the names of species across the tips of the phylogenetic tree 999 times (Webb et al., 2008). Standardized effect size (SES; Gotelli & Graves, 1996) was calculated for taxonomic (β -deviation) and phylogenetic (SES.D_{pw}) β -diversity using the mean and standard deviation of their respective null distributions:

$$SES.X = \frac{X_{observed} - \overline{X_{null}}}{sd(X_{null})} \quad (2.2)$$

where $X_{observed}$ is the observed β -diversity value between two communities, $\overline{X_{null}}$ is the mean value of the null distribution and $sd(X_{null})$ is the standard deviation of the null distribution. Positive and negative values indicate higher and lower β -diversity than expected by chance, respectively, whereas a value of zero indicates that the observed β -diversity does not differ from random patterns.

2.2.4 *Species colonization and extinction*

We classified a species to be locally extinct from a plot if they were present in 2005 but absent in 2013, and defined new colonists as species that were absent in 2005 but present in 2013 in a plot. To examine the effect of new colonists on the taxonomic and phylogenetic dissimilarity among plots within a treatment, we calculated the average β -

deviation and phylogenetic SES.D_{pw} between new colonists in each replicate plot and final species composition in the three other replicate plots under the same treatment (β -deviation(C) and SES.D_{pw}(C)). A negative β -deviation(C) or SES.D_{pw}(C) indicates that new colonists in a plot are more similar or phylogenetically more closely related to the species composition in other plots of the same treatments than expected by chance respectively. To examine the effect of the extinct species on the taxonomic and phylogenetic dissimilarity among plots within a treatment, we calculated the average β -deviation and phylogenetic SES.D_{pw} between extinct species in each replicate plot and the final species composition in the other replicate plots under the same treatment (β -deviation(E) and SES.D_{pw}(E)). For species extinction, a positive β -deviation(E) or SES.D_{pw}(E) indicates that the extinct species in a plot are more dissimilar or phylogenetically more distantly related to the remaining species in other plots of the same treatments than expected by chance respectively. To address the possibility that the results based on 2005 and 2013 only may be vulnerable to observation error, we also divided the experiment into three periods (2005–2007, 2007–2010 and 2010–2013), and analyzed β -deviation(C/E) and SES.D_{pw}(C/E) for each period. Consistent results between the three periods would lend greater credibility to our results.

To assess the contributions of species' functional traits on the pattern of SES.D_{pw}, we calculated SES.D_{pw} values of functional traits for both colonist and extinct species using the dendrograms of the measured functional traits (i.e. plant height, rooting depth, leaf N concentration and SLA). We generated four trait dendrograms, one for each functional trait,

using UPGMA clustering based on the Euclidean distance matrix (Petchey & Gaston, 2002).

2.2.5 *Statistical analysis*

To test for the effects of N and water addition on taxonomic and phylogenetic β -diversity and their standardized effect sizes over time, we conducted a permutational multivariate analysis of variance (PERMANOVA; 999 permutations; Anderson, 2001) in which fertilization, watering, time and their interactions were used as explanatory variables. Following PERMANOVA, we also used permutational analysis of multivariate dispersions (PERMDISP) to test whether communities differ in their within-treatment dissimilarities (Anderson, 2006; Anderson et al., 2006).

We calculated the phylogenetic signal of the four functional traits measured in this study using the K statistic (Blomberg et al., 2003). The significance (p-values) of the phylogenetic signal was evaluated by comparing the variance of independent contrasts for each trait to the expected values obtained by shuffling leaf trait data across the tips of the phylogenetic tree 999 times. To assess the importance of species' initial coverage and functional traits on colonization and extinction, we ran logistic regressions of species colonization/extinction as a function of species' initial coverage and trait values (i.e. plant height, rooting depth, leaf N concentration and SLA). Species that did not colonize or go extinct in any plot within a treatment were assigned a value of 0. Otherwise, species were assigned a value of 1. We assigned values for colonization and extinction separately.

All analyses were performed using R 3.5.1 (R Core Team., 2018). The Bray-Curtis index was calculated using the *vegdist* function in the package *vegan* (Oksanen et al., 2018), and D_{pw} was calculated using the *comdist* function in the package *picante* (Kembel et al., 2010). The null communities were generated using the *nullmodel* in the *vegan* package (Oksanen et al., 2018). PERMANOVA and PERMDISP were performed using the functions *adonis2* and *betadisper* in the *vegan* package respectively (Oksanen et al., 2018). The analyses on phylogenetic signal were conducted using the function *multiPhylosignal* in the *picante* package (Kembel et al., 2010).

2.3 Results

2.3.1 Species taxonomic and phylogenetic β -diversity, and their standardized effect size

Taxonomic β -diversity (Bray-Curtis index) fluctuated significantly over time in all but the water addition treatments. Taxonomic β -diversity exhibited a positive trend only in the N addition treatment, resulting in greater β -diversity in this treatment than the controls (Figure 2.2a). Phylogenetic β -diversity (D_{pw}) remained largely unchanged in the N addition and N + water addition treatments, but declined over time in the control and water addition plots, resulting in greater phylogenetic β -diversity in the N addition and N + water addition plots towards the end of the experiment (Figure 2.2c). PERMANOVA indicated that N enrichment, water addition, year and all their two-way interaction terms significantly affected taxonomic β -diversity, whereas phylogenetic β -diversity was only significantly affected by N addition (

Table 2.1). PERMDISP revealed that N, water and N + water addition treatment increased the dispersion of species composition (Figure B. 2).

β -deviation showed a similar temporal pattern as the Bray-Curtis index. At the end of the experiment, β -deviation was not significantly different from null expectation in the control plots, but was significantly higher than null expectation in the N, water and N + water addition treatments, with the highest values observed for the N addition plots (Figure 2.2b). The standardized effect size of D_{pw} (i.e. $SES.D_{pw}$) was significantly greater than zero in all treatments initially (one-sample t-test, $p < 0.05$). At the end of the experiment, however, $SES.D_{pw}$ was significantly negative (one-sample t-test, $p = 0.018$) in the water addition plots and not significantly different from zero in the other treatments (one-sample t-test, $p > 0.05$; Figure 2.2d). Correspondingly, PERMANOVA indicated that N enrichment, water addition and their interaction terms significantly affected β -deviation and that $SES.D_{pw}$ was only affected by water addition (

Table 2.1). PERMDISP revealed that D_{pw} showed greater dispersion in the N, water and N + water addition treatments than the controls, whereas SES. D_{pw} showed lower dispersion in the water addition treatment than other treatments (Figure B. 2; Table B. 1).

Table 2.1 – Results of permutational multivariate analysis of variance (PERMANOVA) on the effects of N enrichment, water addition, year, and their interactions on community taxonomic β -diversity (Bray-Curtis), phylogenetic β -diversity (D_{pw}), and their respective standard effect sizes (β -deviation and SES. D_{pw}). The analyses were performed using 999 permutations.

	Df	Bray-Curtis		D_{pw}		β -deviation		SES. D_{pw}	
		F	p	F	p	F	p	F	p
N	1	20.45	0.001	11.62	0.001	1.79	0.001	0.87	1.000
Water	1	5.21	0.001	1.17	0.181	1.15	0.001	1.02	0.035
Year	1	12.42	0.001	1.55	0.055	1.00	0.467	0.98	0.924
N:W	1	2.69	0.016	1.10	0.212	1.08	0.032	0.99	0.858
N:Y	1	3.69	0.002	1.63	0.047	1.16	0.003	1.00	0.614
W:Y	1	2.66	0.011	1.30	0.113	1.10	0.012	1.01	0.110
N:W:Y	1	1.02	0.382	0.92	0.499	1.00	0.500	0.99	0.765

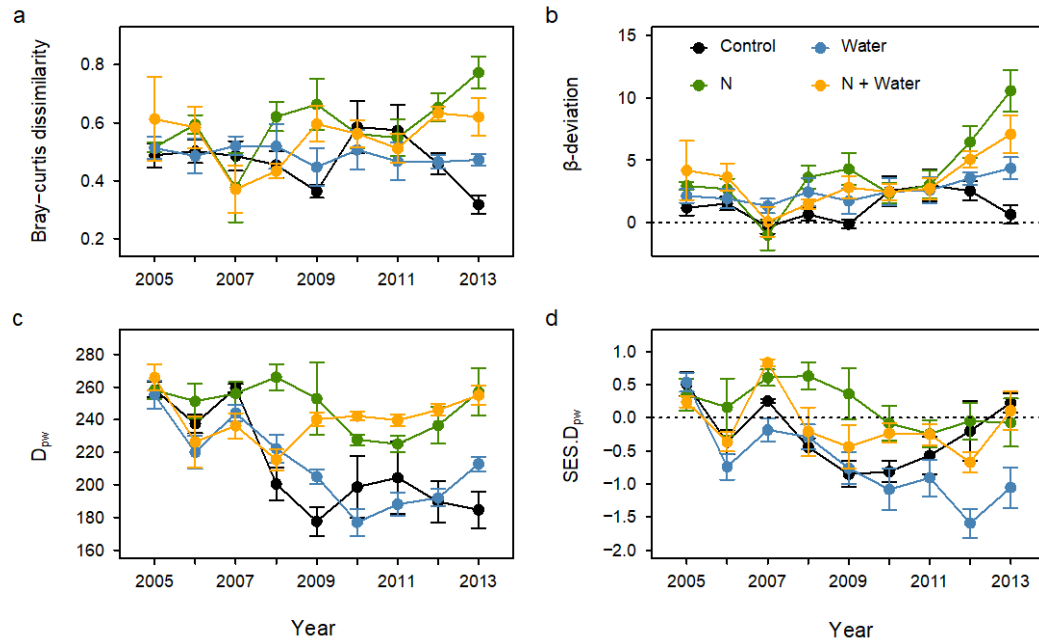


Figure 2.2 – Changes in taxonomic β -diversity, phylogenetic β -diversity, and their respective standardized effect sizes among replicate plots within each treatment over time. Taxonomic β -diversity and its standardized effect size were measured by (a) Bray-Curtis dissimilarity and (b) β -deviation, respectively. Phylogenetic β -diversity and its standardized effect size were measured by (c) D_{pw} and (d) $SES.D_{pw}$, respectively. Values are mean \pm standard error. The standardized effect sizes (β -deviation and $SES.D_{pw}$) show the magnitude of deviation between observed β -diversity and the values generated from null models. Negative values indicate lower β -diversity than expected from chance, while positive values indicate the opposite.

2.3.2 *Species colonization and extinction*

In the control plots, most of the extinct species were forbs from the genera *Allium* and *Potentilla* and most colonists were grasses from the family Poaceae. In plots with N fertilization, in addition to species that went extinct in the controls, some grasses from the family Poaceae and Cyperaceae also went extinct; the few colonists were mainly forbs from the families Rosaceae and Cruciferae. In the water addition plots, however, the number of extinct species was much lower, with most being extinct also in the control plots. Species colonizing the water addition plots were mainly forbs from the families Labiatae, Gentianaceae and Leguminosae (Figure B. 1).

The compositional dissimilarity between colonists and final community composition within a treatment (i.e. β -deviation(C)) was significantly greater than zero in all treatments, indicating that colonists were more dissimilar to species in other replicate plots than null expectation (Figure 2.3a; one-sample t-test, $p < 0.05$). The compositional dissimilarity between extinct species and the final community composition within a treatment (i.e. β -deviation(E)) was also significantly greater than zero in all treatments (Figure 2.3b; one-sample t-test, $p < 0.05$), indicating taxonomically deterministic extinction. The phylogenetic SES. D_{pw} between colonists and final community composition within a treatment (i.e. SES. D_{pw} (C)) did not significantly differ from zero in the control, N addition and N + water addition treatments (Figure 2.3c; one-sample t-test, $p > 0.05$), indicating phylogenetic randomness of species colonization in these treatments. However, we found significant negative phylogenetic SES. D_{pw} (C) in the water addition treatment (Figure 2.3c; one-sample t-test, $p = 0.035$), indicating that colonizing species in each water

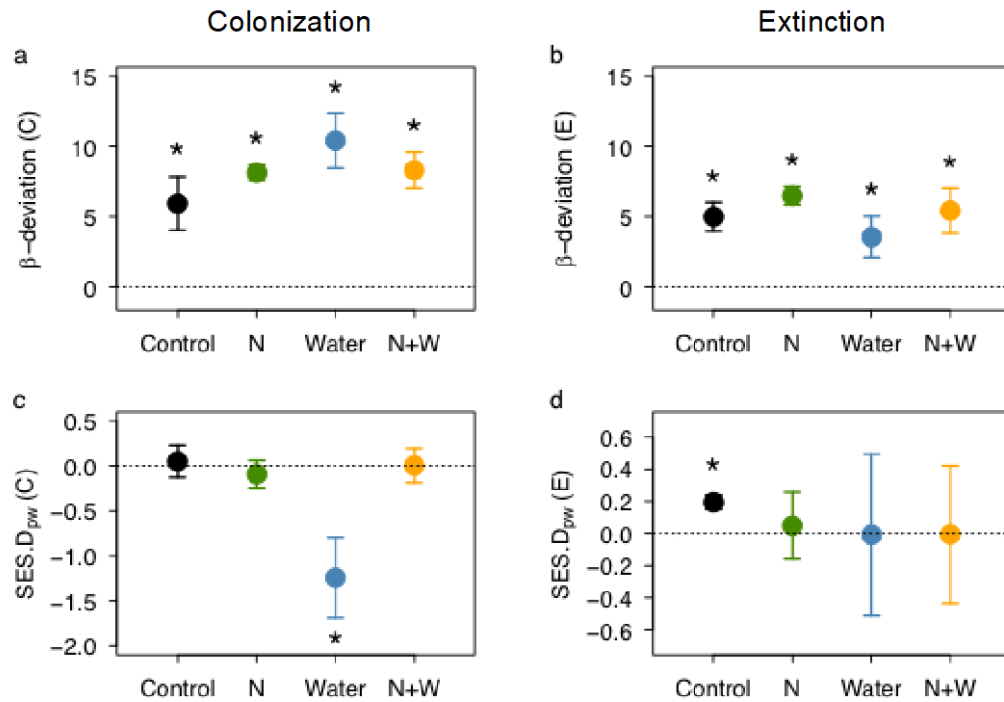


Figure 2.3 – The taxonomic (β -deviation) and phylogenetic dissimilarity (SES.D_{pw}) between new colonists and extinct species of each plot and final species composition in the other three replicate plots within the same treatment. For species colonization, a negative β -deviation(C) indicates that new colonists are more similar to the final communities in other replicates than expected by chance, and a negative SES.D_{pw}(C) indicates that new colonists are more phylogenetically closely related to the final communities in other replicates than expected by chance, indicating deterministic colonization. For species extinction, a positive β -deviation(E) indicates that extinct species are more dissimilar to the remaining species in other replicates than expected by chance, and SES.D_{pw}(E) indicates that extinct species are more phylogenetically distantly related to the remaining species in other replicates than expected by chance, indicating deterministic extinction. * denotes values that are significantly different from zero based on one-sample t test ($p < 0.05$). Error bars represent standard errors.

addition plot were more closely related to species in other replicate plots than expected by chance. The phylogenetic $SES.D_{pw}$ between extinct species and final community composition within a treatment ($SES.D_{pw}(E)$) was not significantly different from zero in the N, water and N + water addition treatments (Figure 2.3d; one-sample t-test, $p > 0.05$), indicating phylogenetic randomness of species extinction in these treatments. The average phylogenetic $SES.D_{pw}(E)$ in the controls was significantly greater than zero (Figure 2.3d; one-sample t-test, $p = 0.011$), indicating that extinction excluded species that were more phylogenetically distantly related to the final species composition than expected by chance in this treatment. When the experiment was divided into three periods (2005–2007, 2007–2010 and 2010–2013), the patterns for β -deviation(C/E) and $SES.D_{pw}(C/E)$ within each period were similar to those across all years (Figure B. 3).

Among the four functional traits measured in this study, significant phylogenetic signal was detected only for leaf N concentration ($p = 0.035$, Table B. 1). Therefore, we presented the results on leaf N concentration in the main text and the results on other functional traits in the supporting information (Figure B. 4; Figure B. 5; B.1; B.2). The $SES.D_{pw}(C)$ for leaf N concentration showed a similar pattern with phylogenetic $SES.D_{pw}(C)$, such that colonizing species in each water addition plot were more similar in leaf N concentration with species in other replicate plots than expected by chance (Figure 2.4a, one-sample t-test, $p = 0.036$). For species extinction, non-significant $SES.D_{pw}(E)$ for leaf N concentration was found for all treatments (Figure 2.4b).

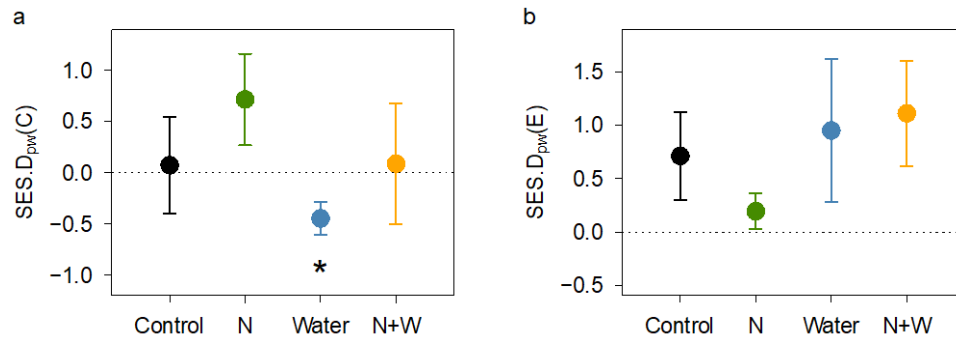


Figure 2.4 – The functional trait dissimilarity (SES.D_{pw}) for leaf N concentration between new colonists and extinct species of each plot and final species composition in the other three replicate plots within the same treatment. A negative SES.D_{pw}(C) indicates that new colonists are more similar to the final species composition than expected by chance. A positive SES.D_{pw}(E) indicates that extinct species are more different from the remaining species than expected by chance. * denotes values that are significantly different from zero based on one-sample t test ($p < 0.05$). Error bars represent standard errors

The initial coverage of species was a significant predictor of species extinction in all treatments. Species with lower initial coverage tended to have a greater probability of extinction (Figure 2.5a-d), corresponding with the taxonomically deterministic extinction found in all treatments (shown in Figure 2.3b). Leaf N concentration affected the likelihood of species colonization in the water addition treatment, such that species with higher leaf N concentrations were more likely to colonize (Figure 2.5g). In the N + water addition treatment, leaf N concentration affected the likelihood of species extinction, such that species with higher N concentration suffered from greater risk of extinction (Figure 2.5h).

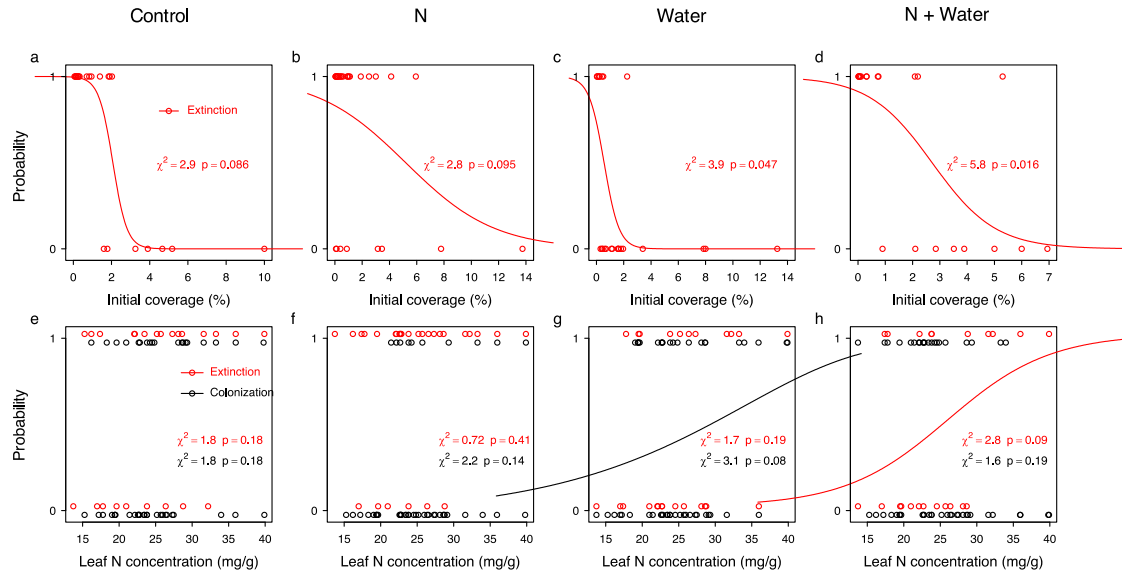


Figure 2.5 – Species colonization and local extinction as functions of initial coverage and leaf N concentration in each treatment. Species that did not colonize or go extinct in any plot within a treatment was assigned a value of 0. Otherwise, species were assigned a value of 1. Significant logistic regression lines ($p < 0.1$) are shown.

2.4 Discussion

The Earth's ecosystems are facing widespread anthropogenic environmental changes. A key challenge is to elucidate how ecological processes interact with evolutionary processes in influencing diversity patterns across spatial scales in the face of anthropogenic environmental changes. In this study, we investigated the impact of elevated N deposition and precipitation on species taxonomic and phylogenetic β -diversity, and linked species colonization and extinction to the observed β -diversity patterns. We found that both N enrichment and water addition significantly increased taxonomic β -diversity, and N enrichment also significantly increased phylogenetic β -diversity. However, when the differences in local community size were controlled for using null models, both N enrichment and water addition significantly increased the standard effect size of taxonomic β -diversity (i.e. β -deviation), suggesting that resource enrichment led to increased taxonomic divergence; water addition, not N enrichment, significantly decreased the standard effect size of phylogenetic β -diversity (i.e. $SES.D_{pw}$), suggesting that water addition drove communities to converge towards more similar phylogenetic structure.

A number of experiments have assessed the effects of resource addition on taxonomic β -diversity. Chalcraft et al., (2008) synthesized data from 18 N-enrichment experiments along a productivity gradient across North America, and found that N addition promoted β -diversity at low - productivity sites but reduced β -diversity at high-productivity sites, with the threshold productivity around $400 \text{ g m}^{-2} \text{ year}^{-1}$. The positive treatment effects on β -diversity in our experiment are in accordance with this general

pattern, as the productivity at our study site is far below the threshold ($60 \sim 250 \text{ g m}^{-2} \text{ year}^{-1}$, Xu et al., 2018). N and water are known to be the two major limiting resources for our study grassland (Bai et al., 2004; Niu et al., 2010). Our results are thus consistent with the idea that adding limiting resources enhances β -diversity in resource-scarce environments, where strong environmental filtering limits community membership in a largely deterministic manner (Chalcraft et al., 2008; Chase, 2010). Note that in our experiment, both taxonomic β -diversity and β -deviation increased in response to N and water addition, indicating that the observed community divergence after N and water addition was due to the enhanced role of stochastic processes rather than changes in α -diversity. One possible mechanism for the more important role of stochastic assembly processes under resource enrichment is that stronger priority effects may lead to the increased likelihood of multiple community states in more benign environments (Chase, 2003, 2007, 2010). In our study, dispersal was highly stochastic at the species level, as evidenced by the compositional dissimilarity between colonized species in each plot and species in other replicate plots (i.e. β -deviation (C)) being much higher than null expectation in all treatments. Under resource amendment, such stochastic dispersal may have led to high variability in species arrival history and, in turn, strong priority effects, promoting the taxonomic divergence of communities (Chase, 2010; Vannette & Fukami, 2017).

We found that water addition, rather than N enrichment, significantly decreased the standard effect size of phylogenetic β -diversity (SES.D_{pw}), driving the communities from being phylogenetically divergent to being phylogenetically convergent (Figure 2d). Such transition in the water addition treatment could be attributed to the phylogenetically non -

random colonization of species. Specifically, the colonists in each plot after water addition were significantly related to species in other replicate plots (Figure 3c), resulting in phylogenetically similar community composition among plots. This pattern contrasts with the taxonomically stochastic colonization and divergence observed in the water addition plots (see the previous paragraph), supporting our hypothesis that taxonomic and phylogenetic β -diversity may not necessarily respond similarly to environmental changes (Graham & Fine, 2008; Hardy et al., 2012). These results emerged likely because water addition favors certain closely related species with similar traits (e.g. those with similar leaf N content, Figure 4a), resulting in community convergence at the phylogenetic scale, but facilitates the non - deterministic colonization of these species among plots (Figure 3a), resulting in community divergence at the species level. One way to confirm this explanation is to eliminate the stochasticity associated with species colonization by, for example seed addition, which would favor community convergence at both taxonomic and phylogenetic scales. Indeed, a recent study has found that fertilization and water addition into a California grassland reduced plant taxonomic β -diversity when seeds of common species were added to all experimental plots (Eskelinen & Harrison, 2015). On the other hand, our results clearly show that considering both phylogenetic and taxonomic turnover allows a better assessment of the role of deterministic and stochastic processes in shaping ecological communities.

We found evidence for functional trait-based species colonization in the water addition plots. Leaf N concentration, the only plant trait that exhibited significant phylogenetic signal (Table B. 2), was found to be more similar between the colonist in each

plot and the species in other replicate plots in the water addition, but not other treatments (Figure 4a). Correspondingly, the probability of colonization was strongly associated with leaf N concentration, with N - rich species tending to have a greater probability of colonization (Figure 5g). Leaf N concentration is a key functional trait on the “leaf economic spectrum” that relates to plant resource capture and conservation (Wright et al., 2004). N-poor species are generally conservative in resource use and expected to be better at coping with abiotic stress (Coley et al., 1985; Díaz et al., 2016; Reich et al., 1997; Wright et al., 2004). Studies that explored relationships between leaf economic traits and climate have found a general tendency for species inhabiting arid and semi-arid regions to exhibit a more conservative strategy in resource use (Wright et al., 2001). In line with these findings, our result demonstrated that enhanced water supply alleviated abiotic stress and facilitated species on the “acquisitive” end of the leaf economic spectrum to colonize, which resulted in phylogenetic homogenization among water addition plots. Finally, we note that traits of the same plant species may respond to resource amendment, such that they may also differ among experimental treatments (Yan et al., 2015). This possibility, however, would need to be addressed by future studies, as we only quantified plant traits in the controls.

Our study provides, to our knowledge, the first experimental evidence that anthropogenic environmental changes can differentially affect plant taxonomic and phylogenetic β -diversity. Both N enrichment and water addition significantly increased taxonomic β -diversity, whereas water addition, not N enrichment, significantly reduced phylogenetic β -diversity, with the latter attributed to colonizing species in each water

addition plot being more closely related to species in other replicate plots of the same treatment. Our results thus illustrate that although stochastic processes may cause communities to diverge more in species composition under anthropogenic environmental changes, deterministic processes could still produce communities more convergent in phylogenetic community structure. It remains to be seen whether these findings apply to other systems and whether they extend to ecosystem functions. For example, an interesting question to ask next is whether community phylogenetic convergence under precipitation amendment would translate into ecosystem functional convergence.

CHAPTER 3.

**EXPERIMENTAL DEMONSTRATION OF KEYSTONE
COMMUNITIES FOR METACOMMUNITY BIODIVERSITY
AND ECOSYSTEM FUNCTIONING**

Abstract. Local communities within a metacommunity may differ considerably in their contributions to biodiversity and ecosystem functioning. Consequently, it has been suggested that conservation priority should be given to disproportionately important local communities (i.e., keystone communities). However, we know little about what characterizes a keystone community. Using laboratory protist microcosms as the model system, we examined how the environmental uniqueness and location of a local community affect its keystone-ness. We found that the removal of local communities with unique environmental conditions that supported endemic species, reduced regional-scale diversity, qualifying them as regional-scale keystone communities. In addition, the local communities possessing unique environmental conditions had greater impacts on ecosystem functions, including biovolume production and particulate organic matter decomposition. We also found that keystone communities for biovolume production were not keystone for organic matter decomposition, and vice versa. Our study provides the first experimental evidence for keystone communities, highlighting the important role of keystone communities in maintaining biodiversity and functioning of metacommunities.

Keywords: connectivity, heterogeneity, keystone community, metacommunity

3.1 Introduction

The ever-escalating human activities have been driving rapid habitat loss and fragmentation worldwide, posing a serious threat to Earth's biodiversity (Vitousek et al., 1997; Dirzo & Raven, 2003; Fahrig, 2003; Haddad et al., 2015). Habitats in a fragmented landscape, however, are not equal (Myers et al., 2000; Brooks et al., 2006). Analogous to keystone species that play a disproportionally important role, relative to their abundance, in regulating local community structure and ecosystem functioning (Paine, 1966; Power et al., 1996), some communities may be disproportionally more important than others for the maintenance of community and ecosystem properties of the region in which they are embedded, making them the keystone community for the metacommunities (Economo, 2011; Mouquet et al., 2013). The loss of these communities may not only cause the loss of local species diversity but also erode regional biodiversity and ecosystem functions (Mouquet et al., 2013). By contrast, some communities may be considered a burden within metacommunities (Mouquet et al., 2013), and the removal of such burden communities would have a positive effect on community and ecosystem properties of the region. Identifying these keystone/burden communities in a fragmented landscape, therefore, has important implications for the effective allocation of limited conservation resources (Economo, 2011; Mouquet et al., 2013). Nevertheless, empirically we know little about the characteristics of those communities that have large regional impact. The only empirical study on this topic was unable to identify keystone communities within experimental metacommunities (Resetarits et al., 2018).

Metacommunity theory suggests that environmental heterogeneity and patch connectivity are important in determining both local and regional biodiversity (Leibold et al., 2004; Holyoak et al., 2005). We, therefore, hypothesize that the characteristics of local patches, especially their contribution to environmental heterogeneity and patch connectivity, may determine whether the communities in these patches are the keystone communities to the metacommunities. The decrease in environmental heterogeneity due to the loss of a patch with unique environmental conditions may have significant impacts on regional biodiversity. When dispersal rate among local communities is low or moderate, heterogeneity could enhance regional biodiversity through species sorting, by which species differing in their niches utilize different patch types (Leibold, 1998; Chase & Leibold, 2003), or through source-sink dynamics (Pulliam, 1988; Loreau & Mouquet, 1999; Mouquet & Loreau, 2002), by which local populations persist in unfavorable habitats (i.e., sink communities) through migration of individuals from favorable habitats (i.e., source communities) (Mouquet et al., 2006). Under these circumstances, keystone communities are likely to dwell in unique habitat patches that contribute most to environmental heterogeneity and support the positive growth of endemic species populations (Tews et al., 2004; Resetarits et al., 2017). When dispersal rate is sufficiently high, local and regional biodiversity may be depressed as the local communities become homogenized through mass effects (Mouquet & Loreau, 2003). In this situation, habitat patches that support regionally dominant competitors or generalist predators may reduce local and regional diversity through competitive exclusion or predation (Mouquet & Loreau, 2003; Cadotte & Fukami, 2005; Cadotte, 2006). Local communities that support the positive growth of

competitive dominant species or generalist predators, via unique environmental conditions, may therefore be burden rather than keystone communities in metacommunities characterized by strong mass effects.

The contribution of local communities to the spatial configuration, especially the connectivity, of the metacommunities (Altermatt & Holyoak, 2012; Carrara et al., 2014) may also define their keystoneness to the metacommunities. In particular, the loss of a patch that occupies a central position with connections to many other patches would compromise the overall connectivity of a metacommunity. Reduced connectivity could preclude the operation of source-sink dynamics in a metacommunity and, therefore, reduce the number of local patches colonized by certain species (Pulliam, 1988; Loreau & Mouquet, 1999; Mouquet & Loreau, 2002; Thompson et al., 2017). More specifically, when the presence of species in a sink community is maintained through immigration of individuals from source communities, cutting down the migration would increase the risk of local extinction in the sink community, increasing the risk of stochastic extinction across the whole metacommunity. In this situation, highly connected local communities could be qualified as keystone communities. Alternatively, when mass effects dominate the spatial dynamics of the metacommunities with extremely high dispersal rates, communities embedded in high connectivity patches might be burden rather than keystone communities. Removing such local communities may increase regional diversity by maintaining local spatial refuges from competitors and predators (Cadotte & Fukami, 2005).

Removing local habitat patches also may have consequences for ecosystem functioning. On the one hand, ecosystem functions might be affected when the removal of

local patches eliminates the spatial insurance effects of biodiversity, by which ecosystem functions of metacommunities are maintained when species are able to move between patches to track their favorable environments (Loreau et al., 2003a; Gonzalez et al., 2009; Staddon et al., 2010; Shanafelt et al., 2015). The operation of spatial insurance effect requires a combination of species sorting dynamics by which species with different niches are able to disperse to their favorable habitats, and source-sink dynamics by which species could persist in habitats where the environment is unfavorable (Thompson et al., 2017). Both mechanisms could be eliminated when patches that contribute the most towards connectivity and/or heterogeneity are removed. On the other hand, accompanied by the dispersal of organisms, the cross-habitat movements of energy and materials could exert important influences on ecosystem functioning (Polis et al., 1997; Loreau et al., 2003b). In metacommunities where biomass or nutrient moves from productive or fertile patches (i.e., source patches) to unproductive or infertile patches (i.e., sink patches) (Loreau et al., 2003b; Gravel et al., 2010; Mouquet et al., 2013), removing a source patch may reduce ecosystem functions (e.g., primary productivity), while removing a sink patch is likely to enhance ecosystem functions in the neighboring patches or the whole metacommunity (Mouquet et al., 2013). As local patches can be sources or sinks for different materials, and thereby are important for carrying out different functions, focusing on different ecosystem functions might result in different keystone communities. Moreover, loss of keystone communities may impair ecosystem functions indirectly through biodiversity loss, as a large number of studies have reported positive effects of biodiversity on ecosystem functioning (i.e., BEF)

at both local (Naeem et al., 2012; Hooper et al., 2005, 2012; Cardinale et al., 2006, 2012) and regional scales (Venail et al., 2010; Grace et al., 2016).

To examine how the environmental uniqueness and location of a local community affect its keystone-ness, we conducted an experiment using laboratory microcosms consisting of freshwater protozoan communities. In our experiment, each of the metacommunities contained three local communities that included one middle community being lined up with and connected to the other two local communities. We incubated the three local communities under light or dark conditions to manipulate environmental heterogeneity. The loss of one of the local communities would result in changes in environmental heterogeneity and/or patch connectivity of the metacommunities (see Figure 3.1; Figure 3.2). This simple experimental setting allowed us to explicitly test the hypothesis that unique environmental conditions and high connectivity confers keystone-ness in local communities, when considering their influences on the overall species diversity and ecosystem functioning of a metacommunity.

3.2 Materials and methods

3.2.1 Experimental organisms

Our experiment used seven freshwater bacterivorous ciliated protist species, including *Colpidium kleini*, *Dexiotricha granulosa*, *Paramecium bursaria*, *Paramecium caudatum*, *Spirostomum ambiguum*, *Spirostomum teres*, and *Tetrahymena thermophila*.

Among the study species, *Paramecium bursaria* contains endosymbiotic green algae (*Chlorella sp.*) within its cells. Prior to the experiment, we grew each of the study species separately to its carrying capacity in its stock culture.

3.2.2 Experimental design and setup

We used 25 mm × 150 mm Pyrex glass tubes, each of which contained 20 mL of protozoan pellet medium, as the microcosms. We first mixed the protozoan pellets (0.55g/L; Carolina Biological Supply Company, Burlington, NC, USA) in deionized water in a 2 L flask. We sterilized the medium by autoclaving and inoculated three bacterial species: *Bacillus cereus*, *Bacillus subtilis*, and *Serratia marcescens*, as the food for the protists, into the medium. The bacterized medium was incubated at room temperature for three days prior to the experiment. At the beginning of the experiment, we added 20 mL of the medium and 0.2 mL of the stock culture of each of the seven protist species into each microcosm. The initial population size of each species was therefore set as 1% of the carrying capacity of each species. In addition, we added one autoclave-sterilized wheat seed to each microcosm as the extra carbon source. The wheat seeds were oven dried at 75°C for 48h and weighed before the experiment.

Our experiment included six types of metacommunities, each of which contained three microcosms that were lined up with one middle community connected to the other two local communities (Figure 3.1). We considered the protist communities in each microcosm a local community and the assemblage of the three connected microcosms a metacommunity. Under this experimental setting, we can independently control the

environmental heterogeneity and connectivity in each metacommunity. We assigned each microcosm to either the light (red circles in Figure 3.1) or dark (blue circles in Figure 3.1) treatment to create environmental heterogeneity in the metacommunity. Metacommunities that included both light and dark patches had greater environmental heterogeneity than metacommunities that only consisted of one type of patches. All possible combinations of light and dark treatments were included in the experiment (Figure 3.1 A through F).

Each week, we performed dispersal among the three local communities in each metacommunity. We transferred 2 mL (10%) culture from each side patch to the middle patch and then transferred 2 mL medium from the center patch back to each of the side patches. The microcosms were well mixed with a vortex mixer for 10s before each transfer. There was no direct culture exchange between the two side patches. The dispersal rate (equivalent of 1.4% of total populations per day) was comparable with the dispersal rates of zooplankton among hydraulically connected ponds (Michels et al., 2001). To estimate the population density of each protist species, we withdrew a 0.4 mL sample from each microcosm, distributed the medium into eight small drops on a petri dish, and counted the number of individuals of each species in the sample under a stereoscopic microscope. Samples containing large protist populations were diluted before counting. In addition, to replenish resources and remove the metabolic wastes, we replaced 1 mL of the culture with 1.4 mL fresh medium weekly.

All the protist populations reached equilibrium during week 4, when we removed one microcosm from each metacommunity. The loss of one of the local communities could result in the reduction in environmental heterogeneity and/or patch connectivity of the

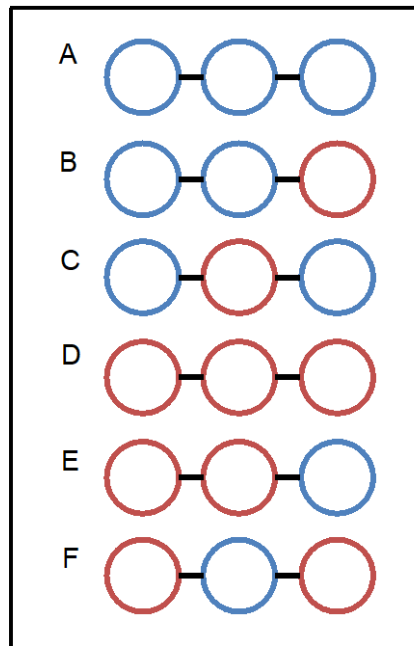


Figure 3.1 – Initial patch composition of metacommunities. Blue and red circles indicate microcosms incubated under dark and light condition, respectively. Black lines indicate dispersal.

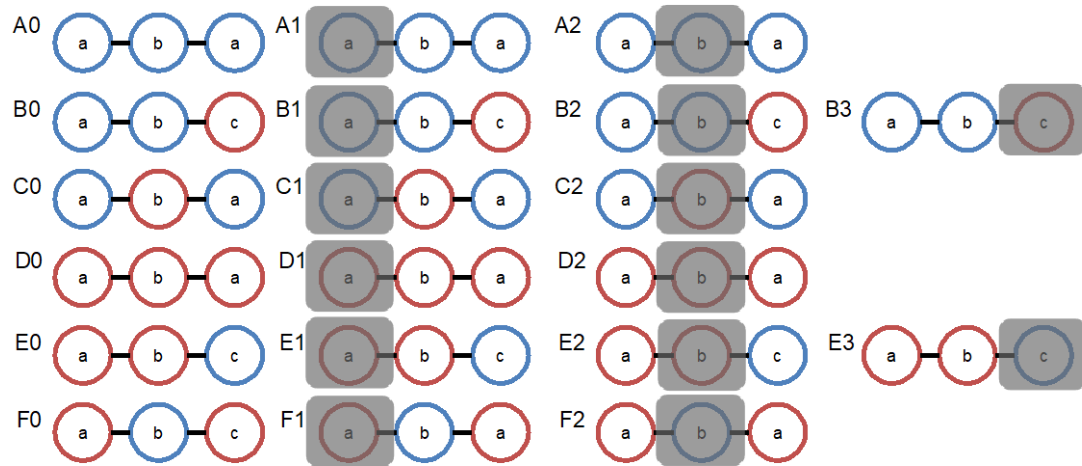


Figure 3.2 – Experimental design. A-F indicate different metacommunities, each of which contains three local communities. Each microcosm was assigned to either the light (red circles) or dark (blue circles) treatment. Black lines indicate dispersal among local communities. Within a metacommunity, equivalent patches are represented by the same lower-case letters. Patches in shade were removed on Week 4. The patch removal reduces the heterogeneity of metacommunities B3, C2, E3, and F2, and reduces the connectivity of metacommunities A2, B2, C2, D2, E2, and F2.

metacommunities. Removing a middle patch would reduce the patch connectivity of the metacommunity. No dispersal was performed between the two side patches when the middle patches was removed. Removing the only light or dark patch would reduce the environmental heterogeneity of the metacommunity. All possible ways of patch removal are shown in Figure 3.2 (A1-2, B1-3, C1-2, D1-2, E1-3, F1-2), including control metacommunities in which none of the local communities were removed (Figure 3.2 A0 through F0). Each combination of metacommunity type and patch removal had three replicates, resulting in 60 experimental metacommunities. After the patch removal, we ran the experiment for another three weeks and terminated it in week 7.

At the end of the experiment, the wheat seed in each microcosm was retrieved, oven dried to constant weight, and weighed. The particulate organic matter decomposition in each microcosm was quantified as the proportion of wheat seed weight loss during the experimental period. Ten individuals of each species from the experimental microcosms were randomly selected and photographed using a digital camera attached to a compound microscope. We measured their cell length and width to estimate the average cell volume of each species based on equations that approximate cell shapes (Wetzel & Likens, 2000). The biovolume production in each microcosm was calculated as the sum of population biovolume of each species.

3.2.3 *Statistical analysis*

We quantified regional (γ -) and local (α -) diversity as species richness of the entire metacommunity and species richness of a single patch, respectively. To identify the

keystone communities for biodiversity and ecosystem functioning, we first quantified the impacts of each local community on the metacommunity properties (e.g., biodiversity and ecosystem functions) through comparing a metacommunity's property with and without patch removal. Changes in regional species diversity were quantified as the difference in γ -diversity between a metacommunity with patch removal and that of the control without patch removal. To identify the communities that affect adjacent local communities disproportionately, we also quantified the impacts of each local community on the properties of other local communities within a metacommunity. Changes in local species diversity were quantified as the difference in α -diversity between a local community in the metacommunity with patch removal and that of its counterpart in the corresponding metacommunity without patch removal. We used similar methods to calculate the changes in two metrics of ecosystem functioning: biovolume production and particulate organic matter decomposition. Regional biovolume production and seed decomposition were calculated as the average values of local respective metrics within a metacommunity.

For a community to be keystone, it needs to have a disproportionate impact on the metacommunity properties relative to its weight (Mouquet et al., 2013). We compared the impacts of local communities relative to their patch size, species total abundance, and biovolume, three metrics that were commonly used as the weight of a community, to ascertain whether their impacts are disproportionate (Mouquet et al., 2013). The patch size, quantified by the volume of medium in each microcosm, was the same across all local patches. We used three-way ANOVA, followed by Tukey's HSD test, to assess the effects of heterogeneity loss, connectivity loss, and the environmental condition of the removed

patch on changes in three community/ecosystem properties (i.e., species diversity, biovolume production, and seed decomposition) at both regional and local scales. If the loss of a metacommunity feature (i.e., heterogeneity or connectivity) significantly reduces (or increases) biodiversity or ecosystem functions, the local communities that contribute to this metacommunity feature would have disproportionately large impacts on the metacommunity relative to their patch size and would be considered a keystone (or burden) community for the property of interest. We also analyzed metacommunities that lost a light or dark patch separately to examine the possible influence of environmental conditions of the removed local communities on changes in community properties.

For the other two weight metrics (species total abundance and biovolume), we first obtained the standardized residuals after regressing the impacts of local communities on their species total abundance and biovolume (Mouquet et al., 2013). We then ran three-way ANOVA to test the effects of heterogeneity loss, connectivity loss, and the environmental condition of the removed patch on the standardized residuals. If the loss of a metacommunity feature (i.e., heterogeneity or connectivity) significantly reduces (or increases) the standardized residuals, the local communities that contribute to this metacommunity feature would have disproportionately large impact on the metacommunity relative to their species total abundance and biovolume, therefore should be categorized as a keystone (or burden) community for the property of interest. All statistical analyses were conducted using R (R core team, 2017).

3.3 Results

ANOVA on changes in regional and local diversity (Figure 3.3; Table C. 1) indicates that the impacts of communities in habitats that contribute to spatial heterogeneity, but not connectivity, were disproportionately large relative to their patch size. At the regional scale, the removal of local communities with unique environmental conditions, which resulted in a decrease in habitat heterogeneity, significantly reduced regional diversity ($p < 0.001$; Table C. 1a; Figure 3.3a). However, this effect significantly depended upon the environment of the removed patch ($p < 0.001$; Table C. 1a). Removing the only light patch significantly reduced regional-scale species diversity ($p < 0.001$; Table C. 1b; Figure 3.3b), whereas losing the only dark patch, which also resulted in a decrease in habitat heterogeneity, did not significantly change regional species diversity ($p = 0.271$; Table C. 1c; Figure 3.3c). The removal of the middle communities of a metacommunity, which resulted in a decrease in habitat connectivity in the metacommunity, did not affect regional diversity ($p = 0.874$; Table C. 1a; Figure 3.3a). At the local scale, both heterogeneity loss and connectivity loss significantly reduced the species richness (α -diversity) of remaining local communities ($p = 0.003$ and $p < 0.001$ for heterogeneity loss and connectivity loss, respectively; Table C. 1a). These effects also depended on the environment of the removed patch ($p < 0.001$; Table C. 1a). When separately analyzing the metacommunities losing a light patch and a dark patch, we found that removing a light patch contributing to either habitat heterogeneity or patch connectivity significantly reduced local species diversity ($p < 0.001$; Table C. 1b; Figure 3.3e). On the contrary, the removal of the only dark patch that did not influence habitat connectivity of the

metacommunities increased species diversity, resulting in a significant heterogeneity loss \times connectivity loss term ($p < 0.001$; Table C. 1c; Figure 3.3f). Qualitatively similar results were obtained when using total abundances and biomass as weight metrics (Table C. 3).

Heterogeneity loss and the environment of the removed patch interactively affected regional and local ecosystem functions ($p < 0.001$; Table C. 2a; Figure 3.4). Removing the only light patch significantly reduced regional and local scale biovolume production ($p = 0.003$ and $p < 0.001$ for regional and local scale biovolume production, respectively; Table C. 2b; Figure 3.4b,e), but increased regional and local scale wheat seed decomposition ($p < 0.001$; Table C. 2b; Figure 3.4h,k). These effects, however, were no longer significant when the effects of biovolume of the removed patch were controlled (Table C. 4b). Losing the only dark patch, which also resulted in a decrease in habitat heterogeneity, increased regional and local biovolume production ($p = 0.023$ and $p < 0.001$ for regional and local scale biovolume production; Table C. 2c; Figure 3.4c,f) but did not significantly change wheat seed decomposition ($p = 0.998$ and 0.638 regional and local scale wheat seed decomposition; Table C. 2c; Figure 3.4i,l). However, neither regional nor local scale ecosystem functions were affected by the removal of the middle communities of a metacommunity (Table C. 2; Figure 3.4a-f).

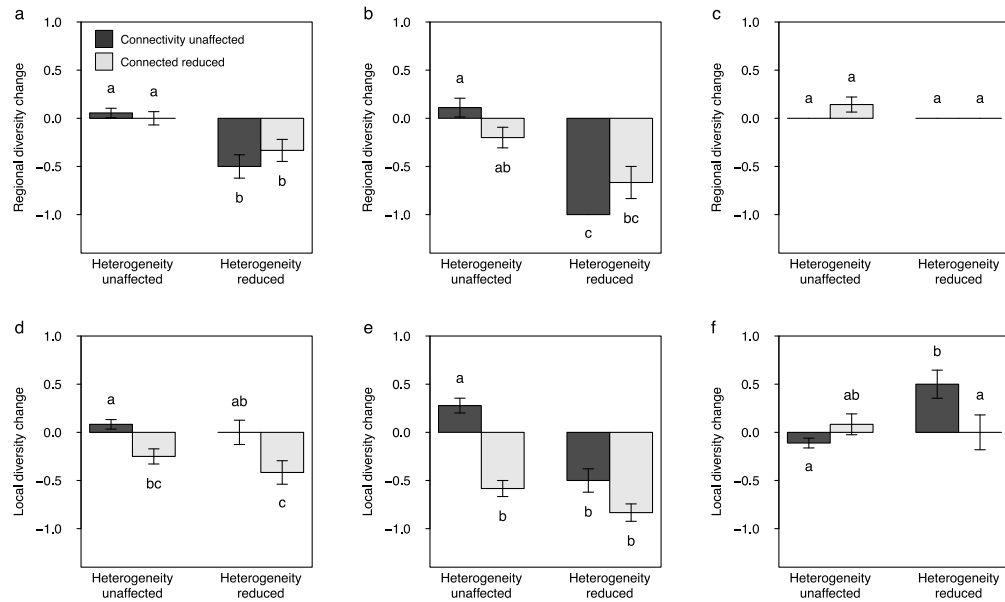


Figure 3.3 – Effects of heterogeneity loss and connectivity loss on changes in regional and local species richness. (a and d), all metacommunities pooled; (b and e), metacommunities losing a light patch; (c and f) metacommunities losing a dark patch. Values are mean+SE. Different letters indicate significant difference ($P < 0.05$) among treatments according to Tukey's HSD tests.

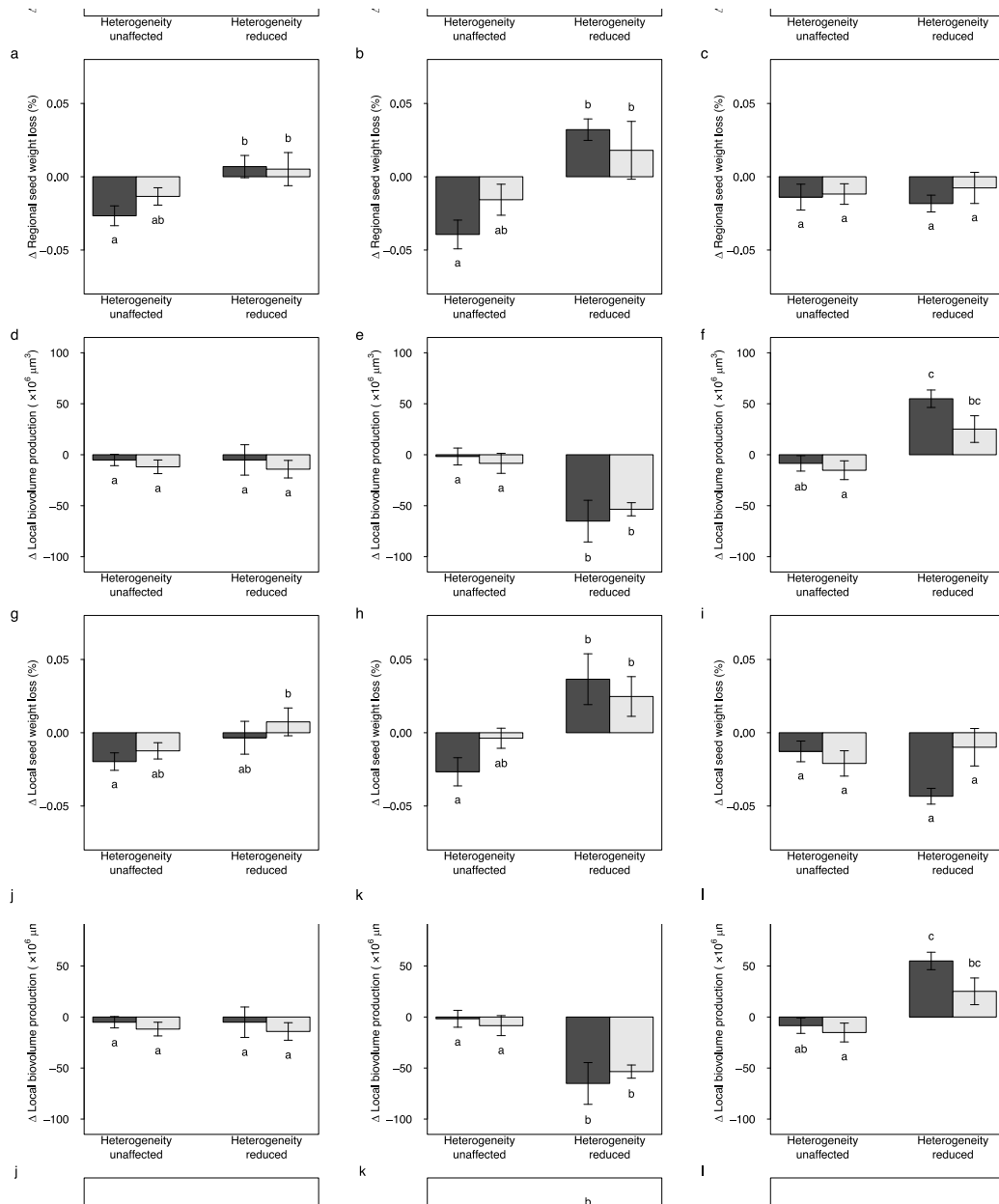


Figure 3.4 – Effects of heterogeneity loss and connectivity loss on changes in regional and local average biovolume production, and on changes in regional and local wheat seed decomposition of metacommunities. (a, d, g, j), all metacommunities pooled; (b, e, h, k), metacommunities losing a light patch; (c, f, i, l) metacommunities losing a dark patch. Values are mean+SE. Different letters indicate significant difference (P < 0.05) among treatments according to Tukey's HSD tests.

3.4 Discussion

The use of “keystone” as a metaphor for ecological concepts dates back to Robert Paine’s classic study on rocky intertidal communities where the predatory starfish *Pisaster ochraceus* helps maintain prey diversity (Paine, 1966; 1969). Since then, this metaphor has been used for species at many other trophic levels, including prey, mutualists, and hosts, with well-described examples (reviewed by Mills et al. (1993) and Power et al. (1996)). The concept of keystone community, recently scaled up from the concept of keystone species, has received little attention since its inception (Economo, 2011; Mouquet et al., 2013). The only empirical exception is Resetarits et al. (2018), who attempted to identify keystone communities by removing local patches at four different locations in 36-microcosm protist metacommunities. However, they found no effect of the patch removal on the biodiversity and biovolume production of the metacommunities. Our experiment showed that the contributions of local communities to environmental heterogeneity and spatial connectivity determined their keystone-ness in a metacommunity. Our study produces three novel findings. First, from the regional-scale perspective, keystone communities are those local communities containing endemic species by virtue of possessing unique environmental conditions. Removing such communities significantly reduced regional-scale biodiversity. Second, from the local-scale perspective, local communities that harbored endemic species and contributed to either environmental heterogeneity or patch connectivity had large impacts on other local communities within a metacommunity. Removing such communities significantly reduced species diversity in adjacent local communities. Third, removing keystone communities significantly altered

ecosystem functions at both local and regional scales. However, the patches that were keystone for certain ecosystem functions could be burden for other functions. We discuss these results in detail in the following paragraphs.

Theory suggests that a local community with particular patch quality would contribute to habitat heterogeneity and may constitute the keystone community to the metacommunity (Mouquet et al., 2013). Such keystone communities could support species that otherwise would not persist in other local communities (i.e., endemic species), making it critical for the species to persist regionally. In line with this theory, we found that the removal of the only light patch caused the largest decline in both regional diversity (Figure 3.3b; Figure 3.5a) and the local diversity of the remaining patches (Figure 3.3e; Figure 3.5b). This pattern is associated with the fact that *P. bursaria*, the species characterized by a mutualistic relationship with the symbiotic green algae, *Chlorella*, coexisted with other species under light conditions but was unable to persist under dark conditions. Several observational studies have suggested the presence of such keystone communities in nature. For example, temporary wetlands in an agricultural landscape in northeast Germany, featured by higher water availability than other parts of the landscape, had higher diversity of carabid beetles by favoring a group of wetland-specific beetles and supporting higher diversity in plant species (Brose, 2003ab; Tews et al., 2004). Another example concerns bird species in the Andaman Islands, India, where the key determinant of regional bird diversity was habitat type, particularly the presence of species-rich wet forests, the reservoirs of many habitat specialists (Davidar et al., 2001). In both examples, communities in those key habitat patches should receive high conservation priority and could be

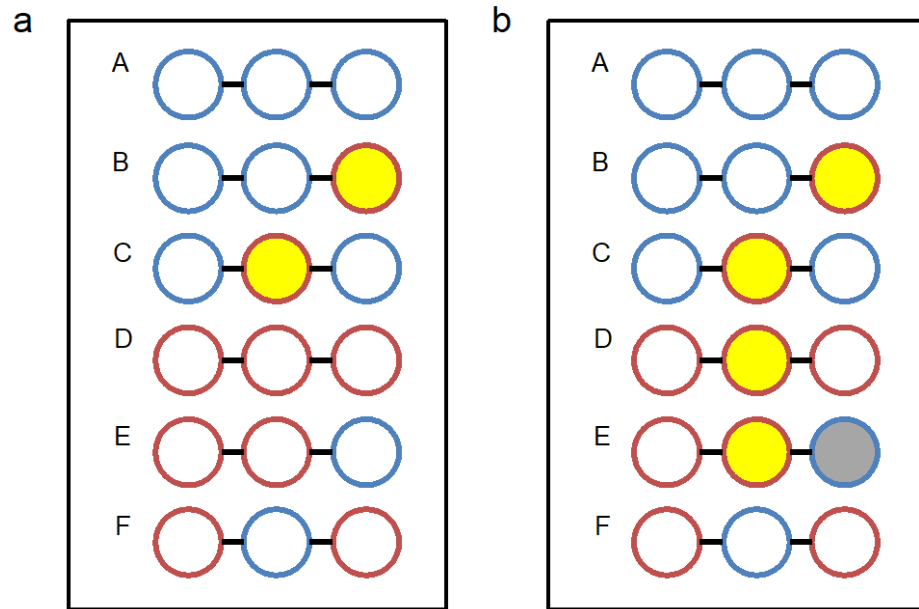


Figure 3.5 – Keystone and burden communities affecting regional and local species diversity. (a) Keystone communities affecting regional species diversity are highlighted in yellow. (b) Communities that positively and negatively affect local species diversity of remaining local communities are highlighted in yellow and gray, respectively.

considered keystone communities; further work is nevertheless needed to demonstrate that their effects on regional biodiversity is disproportionately important. However, counterexamples have been predicted by models with neutral assumptions (Economo, 2011; Gascuel et al., 2016). Economo (2011) used neutral metacommunity models to show that removing patches with high complementarity (i.e., patches that are composed of more endemic species and contribute more to gamma diversity) has no effect on diversity in a metacommunity that is driven solely by spatial structure. Our results suggest that, for non-neutral metacommunities influenced by both patch quality and spatial structure, which are more likely to be common in nature, high complementary patches that harbor endemic species are more important than those with high similarity to other patches in maintaining biodiversity. We also found evidence for burden communities in our experiment. The removal of the only dark patch from a light-dominant metacommunity significantly enhanced species diversity of the remaining light patch, as *P. caudatum* was absent from some light patches before patch removal but was able to persist in all light patches after the dark patch was removed. This result emphasizes the importance of improving or restoring environment in low-diversity patches for conserving regional diversity due to their negative impact on the diversity of neighboring patches (Rey Benayas et al., 2009; Palmer et al., 2016).

Contribution to connectivity is another potentially important feature that influences the keystone-ness of local communities in a metacommunity, as manifested by both theoretical (Muneepeerakul et al., 2008) and experimental (Carrara et al., 2012, 2014) studies reporting that dispersal constrained by habitat connectivity can be a major

determinant of the observed diversity patterns in metacommunities. However, in our experiment, the removal of connectivity had strong negative effects on the local-scale species diversity only when removing a light patch (Figure 3.3e), where dark patches that lost their connection to light patches showed declined species diversity due to the loss of source-sink dynamics. By contrast, removing a middle dark patch did not reduce species diversity in the remaining patches. Our results thus suggest that whether a community contributing to connectivity could be categorized as a keystone community would depend on its local environmental conditions. Similar patterns were found in the study of the range expansion of black woodpeckers in forest patches in Catalonia (Saura et al., 2014). In that study, the amount of habitat that the black woodpecker could reach was primarily determined by the presence of stepping-stone patches with high resource availability; stepping-stones with scarce resources, however, did not promote the dispersal of the black woodpecker. We note that the patches providing connectivity did not contribute much to the maintenance of the regional-scale diversity in our experiment. This result does not mean that these stepping-stone patches are not important in the conservation of regional biodiversity, because local extinction and lack of dispersal would reduce the rescue effect and colonization rate, and may eventually lead to regional extinction (Fahrig, 2002). This regional-scale impact of losing a local community may become more apparent with time, a phenomenon known as extinction debt (Tilman et al., 1994; Jackson & Sax, 2010).

Biovolume production at local and regional scales declined after removing the only light patch, a pattern similar to that of regional biodiversity. *Chlorella*, the endosymbiotic green algae of *Paramecium bursaria*, grew in the light patches, providing additional source

of organic matter for the protist to subsist on. The only light patch in a metacommunity, though possessing lower biovolume compared with dark patches (Figure C. 2), was featured by higher availability of organic matters over dark patches. The presence of these patches could fertilize other patches through weekly dispersal, and thus qualify as a keystone for regional and local biovolume production. However, the patches that were keystone for biovolume production were burden for seed decomposition, as removing the only light patch in a metacommunity increased seed decomposition rate at both local and regional scales. One possible explanation is that *P. bursaria* may have had little effect on seed decomposition in light patches where it dominated. Protist grazing on bacteria is known to promote the decomposition of organic matter (Wang et al., 2009; Jiang, 2007). However, although substantially contributing to community biovolume production (Figure C. 2), *P. bursaria* may have contributed little to seed decomposition, as its growth depends more upon algal symbionts rather than feeding on bacteria when cultured in light (Gu et al., 2002). Our results indicate that different ecosystem functions might not yield similar keystone-ness. A patch that serves as a keystone for one function could be trivial or even a burden for another function (Mouquet et al., 2013).

Our study provided, to our knowledge, the first empirical evidence for keystone communities. For the maintenance of regional biodiversity, keystone communities are those local communities containing endemic species by virtue of possessing unique environmental conditions. As for local biodiversity, local communities play a keystone role when they contribute significantly to either environmental heterogeneity or patch connectivity. Identifying these keystone communities has important implications for

effectively allocating conservation resources, given that current conservation efforts are far from sufficient to protect all species and ecosystem functions in patchy or fragmented landscapes (Pimm et al., 2014). However, there is no uniform standard of keystone community for ecosystem functions, because different ecosystem functions may yield different keystone communities. It is therefore more challenging to identify keystone communities to safeguard different ecosystem functions and services in conservation practices.

CHAPTER 4.

STOCHASTIC PROCESSES ARE IMPORTANT IN SHAPING PHYLLOSPHERE MICROBIAL COMMUNITIES IN A FRAGMENTED SUBTROPICAL FOREST

Abstract. Aerial leaf surfaces, known as the phyllosphere, constitute an inhabitable environment for numerous microorganisms. However, despite much recent research on phyllosphere microbiomes, mechanisms underlying the structure of phyllosphere microbial communities remain poorly understood. By studying phyllosphere microbiomes in fragmented subtropical forests on the islands of Thousand-Island Lake, China, we found that the assembly of phyllosphere bacterial and fungal communities was highly stochastic. In addition to the dominant structuring role of stochastic processes, we also found evidence of deterministic processes shaping phyllosphere microbial communities. Among the host and island characteristics considered in this study, host plant species identity and spatial distance among islands were the most important factors that influence the structure of phyllosphere bacterial and fungal communities, respectively. Our study highlights the importance of stochasticity, and to a lesser extent, deterministic processes, in structuring phyllosphere microbial communities in fragmented systems.

Keywords: bacteria; community structure; fungi; habitat fragmentation; phyllosphere

4.1 Introduction

The phyllosphere, defined as the external surface of plant leaves, covers a considerable area across the earth's terrestrial ecosystems (Last, 1955; Ruinen, 1956, 1961; Vancher et al., 2016). The phyllosphere, therefore, constitutes an important habitat for microorganisms. Ecologists have had a long history of studying microbial communities inhabiting the phyllosphere (i.e., phyllosphere microbiome). Early studies on the phyllosphere microbiome have demonstrated their importance for plant health and performance through a multitude of mechanisms, including disease protection (Last & Deighton, 1965; Leben, 1965) and nitrogen fixation (Ruinen, 1965; Jones, 1970). Recent studies have shown that phyllosphere microbiome also plays a prominent role in regulating ecosystem functions, such as litter decomposition (Osono, 2006; Voříšková & Baldrian, 2013) and ecosystem productivity (Laforest-Lapointe et al., 2017a). Given these functional significances of phyllosphere microbiome for their associated hosts and ecosystems, it is important to develop a thorough understanding of processes and mechanisms that regulate their community structure. However, we still have a rather rudimentary knowledge of the assembly processes of the phyllosphere microbial communities.

All ecological communities, including the phyllosphere microbiome, are under the influences of both deterministic and stochastic processes. Deterministic assembly processes involve ecological selection driven by non-random, niche-based mechanisms, including abiotic environmental filtering as well as biotic species interactions (Chase & Leibold, 2003). The compositional differences among microbial communities across trees could arise from deterministic processes driven by ecological selection that favors different

microbial species under different abiotic environmental conditions or host plant characteristics. In addition to environmental factors such as climate and UV radiation (Vorholt, 2012; Vacher et al., 2016), recent research has demonstrated host species identity to be a key driver of leaf microbial community structure in various terrestrial ecosystems (Redford et al., 2010; Kim et al., 2012; Lambais et al., 2014; Laforest-Lapointe et al. 2016a,b, 2017a,b). In particular, leaf functional traits that relate to leaf morphology, leaf chemistry, and plant growth are reported to be significantly associated with the structure of phyllosphere microbial communities (Kembel & Mueller, 2014; Kembel et al., 2014). Stochastic processes typically involve dispersal and ecological drift (Bell, 2001; Hubbell, 2001; Vellend 2010; Chase & Myers, 2011). Dispersal of phyllosphere microorganisms could occur through airflows (Morris et al., 2014), raindrops or irrigation water (Morris, 2002), and animal transmission (e.g., herbivorous insects). Ecological drift, or random changes in the abundance of microbial taxa, may play an important role in microbial community assembly especially when selection is weak (Vellend, 2010). Drift could lead to the stochastic extinction of rare species, resulting in variation in community structure among similar environment (Nemergut et al., 2013; Vacher et al., 2016). Such variation could further intensify due to priority effects (Chase, 2003), where earlier colonizing species gain numerical dominance over later colonizing species.

Anthropogenic environmental changes, such as habitat fragmentation, are known to affect Earth's ecosystems (Fahrig, 1997, 2003; Haddad et al., 2015). Previous studies on community assembly in the context of habitat fragmentation have focused on animals (Dormann et al., 2007; Orrock et al., 2011) and plants (Cook et al., 2005; Laurance et al.,

2006a,b; Alexander et al., 2012). However, no previous study, to our knowledge, has examined phyllosphere microbial communities in a fragmented landscape. When forests are fragmented, the influence of dispersal and selection processes could be dramatically altered (Davies et al. 2001; Püttker et al. 2015), resulting in significant changes to the assembly of phyllosphere microbial communities. On the one hand, changes in environmental conditions and host plant communities, as a result of decreased habitat area and increased edge effects, could act as environmental filter, thus increasing the importance of deterministic processes (Püttker et al., 2015). On the other hand, the importance of stochastic processes may also be increased because the spatial isolation among habitat patches could increase dispersal limitation. Moreover, the reduced population sizes and increased disturbance by climatic events in the edge habitats may increase the probability of random extinctions and the importance of ecological drift (Haddad et al., 2015; Püttker et al., 2015). Fragmentation likely modifies community assembly through these mechanisms simultaneously and may increase or reduce the significance of deterministic and stochastic processes, depending on the relative importance of these mechanisms.

Here we report on a study of phyllosphere microbial communities in a fragmented subtropical forest on the islands of the Thousand-Island Lake (TIL), China. The TIL is an artificial lake created after damming in 1959. By surveying the phyllosphere microbiome on ten islands, we aimed to (i) explore the effects of island size and isolation on phyllosphere bacterial and fungal diversity, (ii) determine the relative importance of deterministic and stochastic processes in shaping phyllosphere bacterial and fungal communities, and (iii) investigate the roles of host species and island attributes in

regulating the composition of phyllosphere bacterial and fungal communities in the context of habitat fragmentation.

4.2 Materials and methods

4.2.1 Study site and sampling

This study was conducted in May 2017 at the Thousand-Island Lake (TIL) located in Chun'an County of Zhejiang Province, China (29° 22' N to 29° 50' N and 118° 34' E to 119° 15' E, Figure 1). TIL was formed by the construction of the dam of the Xin'an River Hydropower Station in 1959. The historically continuous landscape was fragmented into 1,078 islands with areas greater than 0.25ha when the water level reaches 108 meters above the sea level. The islands are covered by secondary subtropical forests, dominated by *Pinus massoniana* in the canopy and evergreen broad-leaved plants *Castanopsis sclerophylla*, *Cyclobalanopsis glauca*, and *Lithocarpus glaber* in the subcanopy (Hu et al., 2011).

A cluster of ten islands with minimal level of human disturbance, located in the west central region of TIL, were selected as our study sites (Figure 4.1; Table D. 1). On each island, we sampled leaves from each tree species that we encountered on the island. We also sampled each tree species that we encountered on an adjacent mainland forest. In total, 21 tree species were sampled (Table D. 2). For each host species on an island, three individuals were selected. For each individual, we collected ten leaves from different

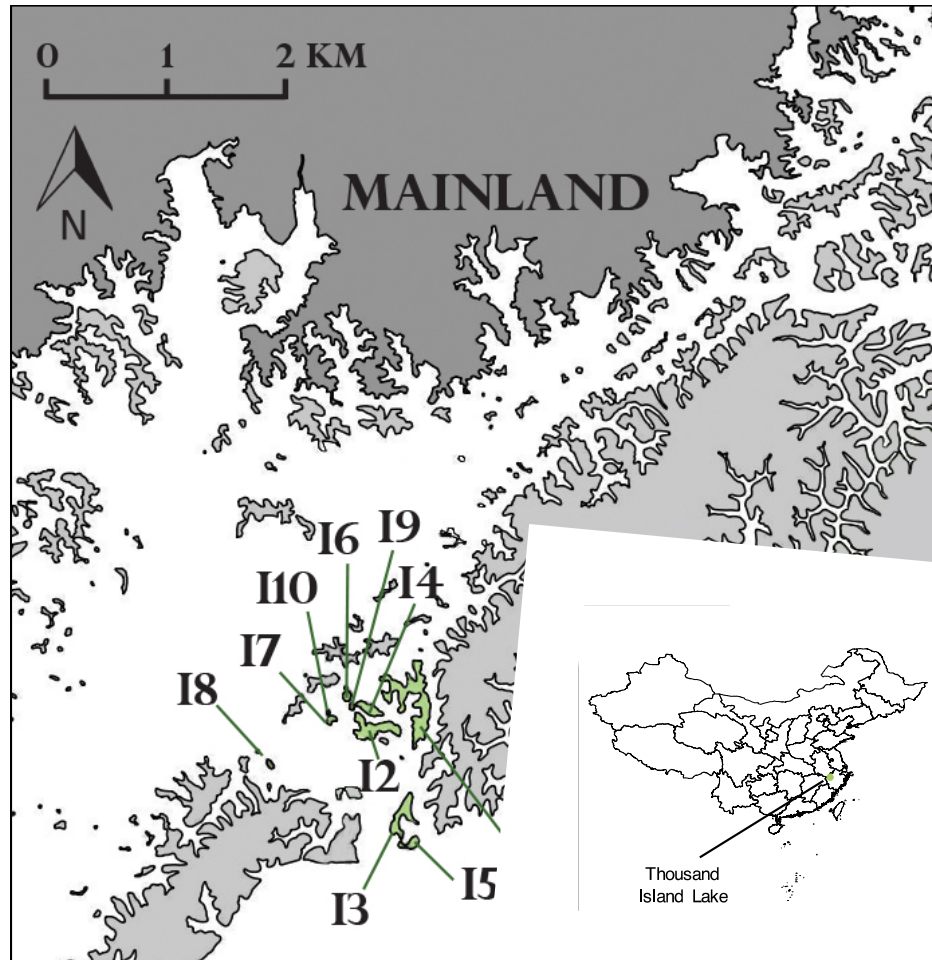


Figure 4.1 – The locations of the 10 study islands in the Thousand Island Lake, and the location of Thousand Island Lake in China. Islands are ranked by descending area. Dark grey area represent mainland by the lake.

branches to formed a composite sample for an individual tree. All samples were placed in sterile sampling bags immediately and transported on ice to the laboratory. We measured three host plant functional traits, including leaf nitrogen (N) concentration, specific leaf area (SLA), maximum plant height (max H), and seed mass (seed M), following the protocol described in Pérez-Harguindeguy et al. (2013).

We collected the microbiome of the leaf surface using sterile polyester-tipped applicators. The applicator was pre-moistened with Potassium Phosphate Buffer solution (0.1 mol/L, pH=7.0), and the whole leaf surface (front and back) was scrubbed with the swab three times with overlapping pattern. After collection, the swab was dipped into sterile Potassium Phosphate Buffer (0.1 mol/L, pH=7.0) within a sterile 1.5mL microcentrifuge tube. All samples were stored at -80°C before DNA extraction.

4.2.2 DNA extraction, sequencing, and bioinformatic analysis

We extracted DNA from microbial cells using the PowerSoil DNA extraction kit according to the manufacturer's instructions. For bacteria, the V4 regions of 16s rRNA gene were amplified by using the primer set: 515 (GTGCCAGCMGCCGCGGTAA) and 806 (GGACTACHVGGGTWTCTAAT) according to the protocol described by Zhou et al. (2016). For fungi, the ITS2 region of the rRNA operon were amplified by using the primer set: ITS3 (GCATCGATGAAGAACGCAGC) and ITS4 (TCCTCCGCTTATTGATATGC) according to Tedersoo et al. (2014). We attached a 12bp barcode unique to each sample to the 5' end of primers 806 and ITS3 to allow multiplexing of samples. PCR products from all samples were then pooled in equimolar concentrations and purified by using the

E.Z.N.A.[®] Gel Extraction Kit. The purified PCR products were subsequently sequenced on a 2×300 bp paired-end Illumina MiSeq platform.

We used software MOTHUR (Schloss, 2009) to process raw reads for quality filtering and assembling. High-quality sequences were assigned to samples according to their unique barcodes and were clustered into operational taxonomic units (OTUs) at the 97% similarity threshold using software USEARCH (Edgar, 2013). Each OTU was classified using the Ribosomal Database Project (RDP) classifier (Wang et al., 2007) according to the SILVA (version 123; Quast et al., 2012) database for bacteria and the UNITE (version 7.0; Abarenkov et al., 2010) database for fungi. OTUs that were not classified into bacteria and fungi were removed before subsequent analyses.

4.2.3 *Statistical analysis*

We rarefied the OTU tables so that all samples matched a uniform sequence depth. We assessed the diversity of phyllosphere microbial communities at both sample- and island-scales. The sample-scale diversity (i.e., alpha diversity) was the estimated OTU richness per sample (i.e., one individual tree). The island-scale diversity was the estimated OTU richness of the whole island. Both alpha and gamma diversity were estimated using Chao 1 index, which is an abundance-based richness estimator that shows high precision when aggregating samples at different scales (Chao, 1987; Hortal et al., 2006). Island isolation was measured as the distance from the island to the mainland. The effects of island area and isolation on bacterial and fungal alpha and gamma diversity were assessed by bivariate regressions.

We quantified the variation in phyllosphere bacterial and fungal community structure among samples using the Jaccard dissimilarity index (Jaccard, 1912). We assessed the relative importance of deterministic and stochastic processes in shaping phyllosphere microbial community structure using the stochasticity ratio (ST). ST is a null-model based index ranging from 0 to 100%; it reflects the contribution of stochasticity relative to determinism based on the magnitude of difference between observed community dissimilarity and null expectations (Ning et al., 2019). We then examined the influence of functional traits of host species, spatial distance among islands, and island area on bacterial and fungal community structures by conducting a permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001). The spatial variables were generated using principle coordinates of neighbor matrices (PCNM) analysis based on distances among islands (Borcard & Legendre, 2002). From all the PCNM axes, we retained those with positive eigenvalues in the PERMANOVA analyses. We also include host species identity and island identity in the PERMANOVA models to account for those host and island attributes that were not captured by the factors we have measured. To illustrate the patterns of bacterial and fungal community structure, we performed a principal coordinate analysis (PCoA) of Jaccard dissimilarities among all samples. All statistical analyses were performed in R 3.5.2 (R Core Team, 2018) with base, vegan (Oksanen et al., 2019), phyloseq (McMurdie & Holmes, 2013), and ecodist (Goslee & Urban, 2007) packages.

4.3 Results

We obtained a total of 19,352,394 and 7,646,549 quality sequences across all samples for bacteria and fungi, respectively. The average number of sequences per sample was $56,752 \pm 35,953$ (mean \pm SD) for bacteria and $19,556 \pm 23,235$ for fungi. After clustering sequences at the 97% similarity level, we detected 10,310 and 3,361 operational taxonomic units (OTUs) for bacteria and fungi, respectively. We rarefied all samples to 8,058 sequences for bacteria and 3,092 sequences for fungi and detected an average of 152 ± 89 bacterial OTUs and 133 ± 38 fungal OTUs per sample. On average, 1,559 bacterial OTUs and 764 fungal OTUs were obtained per island/mainland, and 1,093 bacterial OTUs and 649 fungal OTUs were obtained per host species.

Leaf bacterial and fungal community compositions varied among islands and host tree species (Figure 4.2). Among all samples, two of the eight most abundant bacterial classes belonged to the phylum Proteobacteria, including Gammaproteobacteria (70.01 % of all sequences), Alphaproteobacteria (4.53 %). Two common bacterial classes belonged to the phylum Firmicutes: Bacilli (8.10%) and Clostridia (0.75%). Other classes, including Actinobacteria (7.41%) and Bacteroidia (4.28%), were also abundant (Figure 4.2a,c). Six of the nine most abundant fungal classes belonged to the phylum Ascomycota: Dothideomycetes (46.18% of all sequences), Sordariomycetes (25.48%), Eurotiomycetes (5.87%), Ascomycota cls Incertae sedis (4.61%), Saccharomycetes (2.12%), and Leotiomycetes (1.01%). Three fungal classes belonged to the phylum Basidiomycota, including Tremellomycetes (4.23%), Microbotryomycetes (1.17%), and Agaricomycetes (0.96%), were also abundant (Figure 4.2b,d).

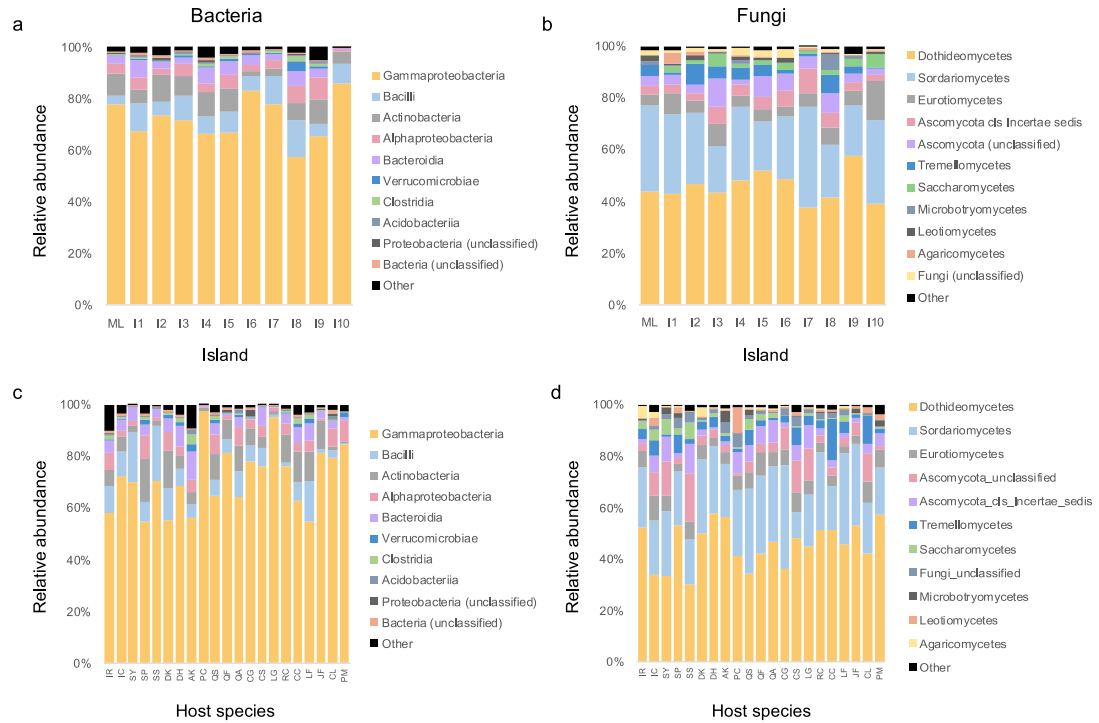


Figure 4.2 – Relative abundance of bacterial and fungal taxonomic classes in the phyllosphere microbiome on different islands/mainland and different host species. Locations of islands represented by the codes are shown in Figure 1. Detailed information of host species is shown in Table D. 2.

Both phyllosphere bacterial and fungal alpha diversity (i.e., OTU richness per individual tree) increased with island area ($R^2=0.037$, $p<0.001$ and $R^2=0.011$, $p<0.001$ for bacteria and fungi, respectively; Figure 4.3a,c), and decreased with island isolation ($R^2=0.047$, $p<0.001$ and $R^2=0.056$, $p<0.001$ for bacteria and fungi, respectively; Figure 4.3b,d). Bacterial and fungal gamma diversity significantly increased with island area ($R^2=0.335$, $p=0.046$ and $R^2=0.592$, $p=0.009$ for bacteria and fungi, respectively; Figure 4.4a,c), whereas neither bacterial nor fungal gamma diversity was related to island isolation (Figure 4.4b,d).

Overall, stochasticity accounted for 72.18% ($ST = 72.18 \pm 17.97\%$) and 80.92% ($ST = 80.92 \pm 15.75\%$) of variation in bacterial and fungal community structure, respectively. Within each island and host species, stochastic processes contributed over 50% of the phyllosphere bacterial and fungal community variation (Figure 4.5).

All factors in the PERMANOVA models significantly influenced phyllosphere bacterial and fungal community structure (Table 4.1). Functional traits and identity of host plants explained 10.64% and 10.68% of the variation in bacterial and fungal community structure, respectively, with most of variation being explained by host species identity ($R^2=8.06\%$, $p=0.001$ for bacteria and $R^2=8.43\%$, $p=0.001$ for fungi; Table 4.1; Figure 4.6). The combination of island area, spatial location, and identity explained 7.44% and 15.25% of variations in bacterial and fungal community structure, respectively, with most variation being explained by spatial location (i.e., PCNM axes) (total $R^2=3.51\%$ for bacteria and $R^2=10.93\%$ for fungi; Table 4.1; Figure 4.6). Island area also significantly affected

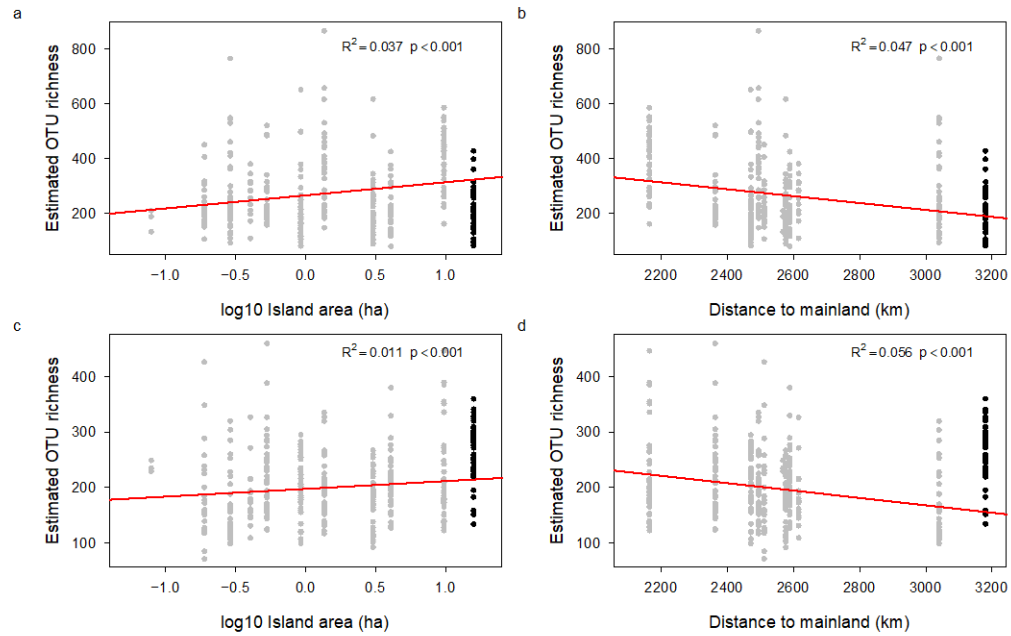


Figure 4.3 – The effects of island area and isolation on the sample-level diversity of phyllosphere bacterial and fungal communities. The sample-level bacterial (a, b) and fungal (c, d) diversity was measured using Chao 1 index. The black dots indicate data from the mainland. The solid lines represent significant linear regressions ($P < 0.001$).

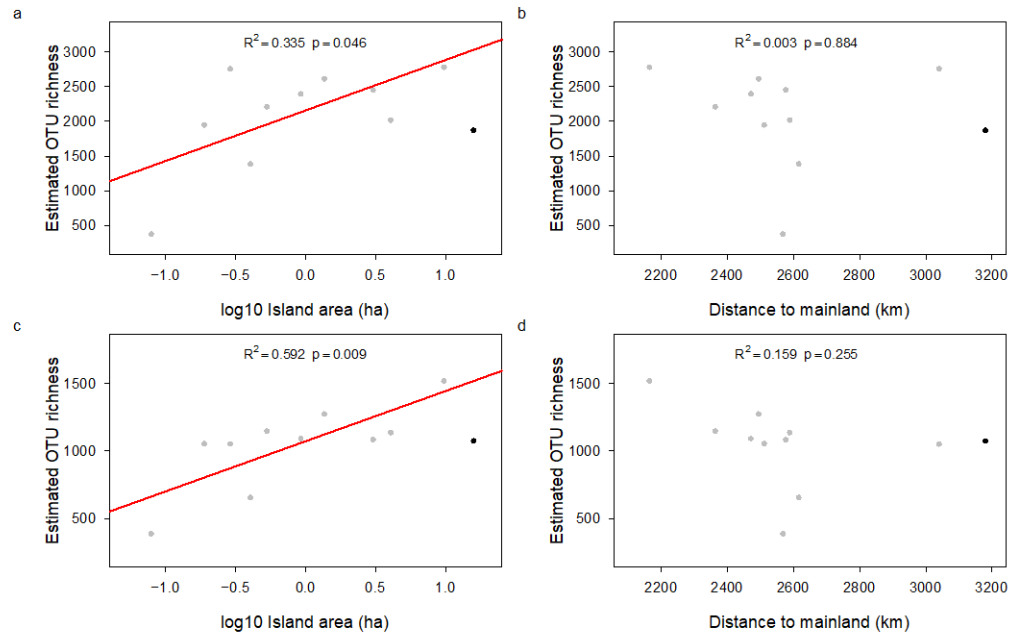


Figure 4.4 – The effects of island area and isolation on the island-level diversity of phyllosphere bacterial and fungal communities. The island-level bacterial (a, b) and fungal (c, d) diversity was measured using Chao 1 index. The black dots indicate data from the mainland. The solid lines represent significant linear regressions ($P < 0.05$).

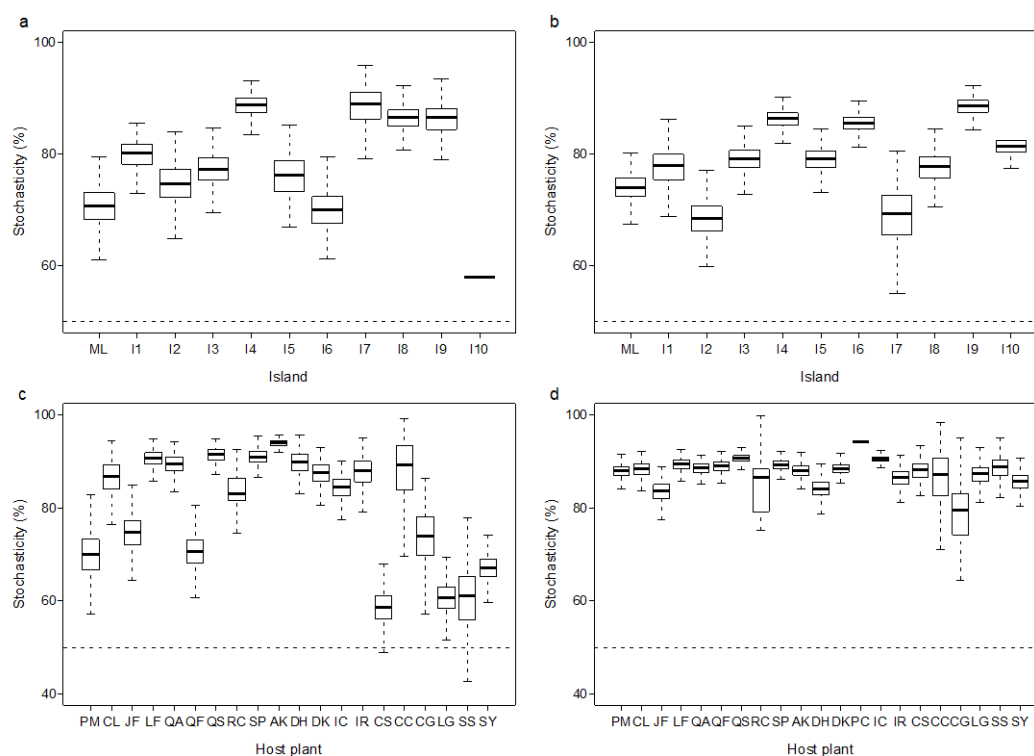


Figure 4.5 – The stochasticity ratio (ST) of phyllosphere bacterial and fungal communities of each island and host plant species. The ST of bacterial (a, c) and fungal (b, d) communities was calculated according to Ning et al., (2019) based on Jaccard index using null model.

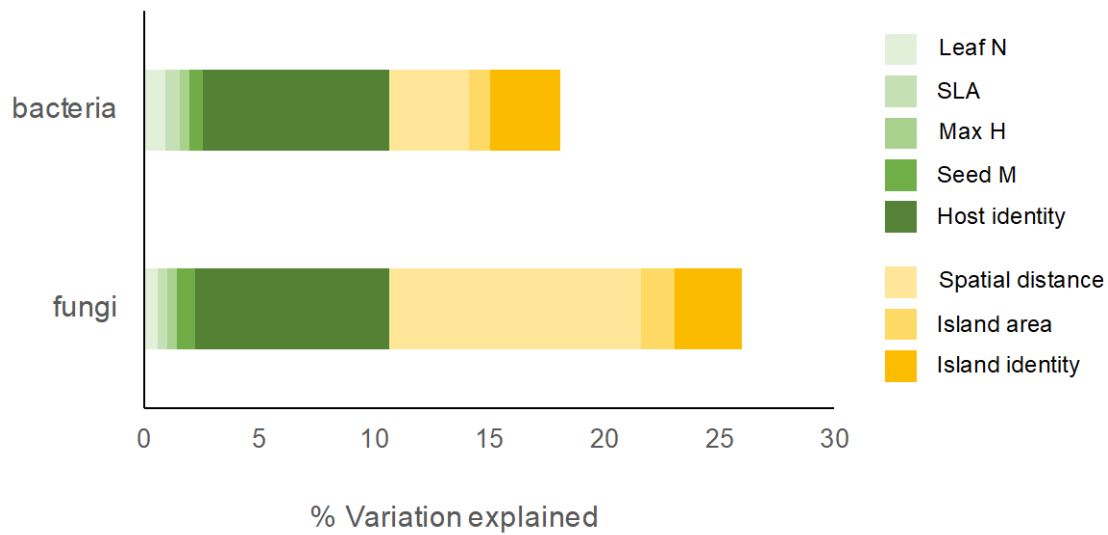


Figure 4.6 – The explanatory power of host plant and island characteristics on phyllosphere bacterial and fungal community structure in the PERMANOVA models.

Table 4.1 – Results of permutational multivariate analysis of variance (PERMANOVA) on the effects of functional traits of host species, spatial distance among islands, and island area on phyllosphere bacterial and fungal community structure. The functional traits of host species include leaf N concentration, specific leaf area (SLA), maximum height (max H), and seed mass (seed M). Spatial distance among islands is represented by PCNM axes. The analyses were performed using 999 permutations.

		Bacteria				Fungi			
		df	F	R ² (%)	p	df	F	R ² (%)	p
Host species	Leaf N	1	3.29	0.92	0.001	1	2.96	0.57	0.001
	SLA	1	2.42	0.68	0.001	1	2.25	0.44	0.001
	Max H	1	1.36	0.38	0.017	1	2.27	0.44	0.017
	Seed M	1	2.14	0.60	0.001	1	4.12	0.80	0.001
	Species identity	15	1.92	8.06	0.001	16	2.72	8.43	0.001
	Total			10.64				10.68	
Island	PCNM1	1	4.53	1.27	0.001	1	15.6	3.02	0.001
	PCNM2	1	3.96	1.11	0.001	1	12.41	2.40	0.001
	PCNM3	1	4.05	1.13	0.001	1	11.73	2.27	0.001
	PCNM4	–	–	–	–	1	8.45	1.64	0.001
	PCNM5	–	–	–	–	1	2.67	0.52	0.001
	PCNM6	–	–	–	–	1	5.59	1.08	0.001
	Island area	1	3.22	0.90	0.001	1	7.39	1.43	0.001
	Island identity	5	2.17	3.03	0.001	2	7.46	2.89	0.001
	Total			7.44				15.25	

bacterial and fungal community structure, but explained less variation comparing with spatial location ($R^2=0.90\%$, $p=0.001$ for bacteria and $R^2=1.43\%$, $p=0.001$ for fungi; Table 4.1; Figure 4.6). Island identity explained 3.03% of the variation for bacterial composition and 2.89% of the variation for fungi composition (Table 4.1; Figure 4.6).

4.4 Discussion

Habitat fragmentation, the division of habitat into small and isolated fragments, is significantly impacting Earth's ecosystems. Reduced fragment area and increased isolation may have the potential to lead to substantial changes in the diversity and composition of ecological communities. In this study, we explored the biogeographical patterns and the drivers of variations in the composition of phyllosphere microbial communities in fragmented subtropical forests on land-bridge islands, and reported three major findings that were not reported previously. First, both sample- and island-scale phyllosphere bacterial and fungal diversity increased with island area. Second, the assembly of phyllosphere bacterial and fungal communities were largely driven by stochastic, rather than deterministic processes. Third, among the host species and island attributes that were considered in our study, host species identity was most important in shaping phyllosphere bacterial communities, whereas island spatial isolation was most important in shaping phyllosphere fungal communities.

The theory of island biogeography proposes that size and remoteness of an island determine its species richness (MacArthur & Wilson, 1967). The positive relationship

observed between island area and microbial richness is consistent with the prediction of this theory. The higher species richness on larger islands may be attributed to several mechanisms. The first hypothesis, known as area *per se* hypothesis, suggested that species have lower extinction risk due to larger population size supported by larger islands (MacArthur & Wilson, 1967; Ricklefs & Lovette, 1999). The second hypothesis, termed habitat-diversity hypothesis, emphasized the importance of higher environmental heterogeneity on larger islands (Connor & McCoy, 1979; Schrader et al., 2019). The third hypothesis, the termed habitat quality effect, focus on the improved environmental conditions on large islands (Schrader et al., 2019). In our study, the sample-scale diversity of phyllosphere bacteria and fungi also increased with island area, which was inconsistency with the prediction of the habitat-diversity hypothesis. Moreover, at the island scale, microorganisms usually have large population sizes and high dispersion rates, and extinctions are expected to be rare (Fenchel, 2003). Therefore, the area *per se* effect might play a minor role in determining the number of microbial OTUs on islands. A more plausible explanation for the observed species-area relationship could be the habitat quality effect. Indeed, comparing with large islands, small islands at our study site are more subjected to the influence of climatic events and harsh environmental conditions such as high UV exposure, which may increase the extinction risk of microbial species.

We found that stochastic processes played predominant roles in shaping phyllosphere bacterial and fungal communities (Figure 4.5). Comparing with soil microbiome whose structure was found to be largely determined by abiotic factors such as soil pH (Rousk et al., 2010; Fiere & Jackson, 2006; Tripathi et al., 2018) and salinity

(Lozupone & Knight, 2007), the assembly of the phyllosphere microbiome is highly stochastic probably for the following reasons. First, leaf surface is subjected to frequent disturbance by climatic events such as wind, rainfall and subsequent splashing of rainwater (Vacher et al., 2016). Such frequent disturbances presumably enhance the stochasticity associated with birth, death, immigration, and emigration (Santillan et al., 2019). Moreover, once the communities were disturbed, the difference in community structure generated by various stochastic processes would be intensified through priority effects (Chase, 2003; Vacher et al., 2016). Second, habitat fragmentation increases edge effects, and the environments in the edge habitats are generally characterized by greater UV exposure and greater wind speed and strength (Davies-Colley et al., 2000; Fahrig, 2003; Grimbacher et al., 2008). These changes in microclimate would further increase the level of disturbance and the role of stochasticity in shaping phyllosphere microbial communities, especially on small islands (Figure 4.5).

Compared to stochastic processes, deterministic processes explained a relatively small fraction of variation in the structure of phyllosphere bacterial and fungal communities. However, the effects of functional traits and identity of host plants in influencing the structure of phyllosphere bacterial and fungal communities are statistically significant (Table 4.1). A number of previous studies conducted in natural continuous habitats have reported that host plant identity was the strongest determinant of tree phyllosphere microbial community structure (Redford et al. 2010; Kembel et al. 2014; Laforest-Lapointe et al., 2016a,b, 2017a,b), and that phyllosphere bacterial (Kembel et al., 2014) and fungal (Kembel & Mueller, 2014) community structure was mainly correlated

with “leaf economic traits” (Wright et al., 2004) that related to resource utilization strategies, such as leaf N concentration and leaf mass per area. In line with these studies, our result also detected significant influence of host plant functional traits on bacterial and fungal community structure, but they explained little variation (Table 4.1). For both bacteria and fungi, host species identity explained more variation than functional traits, indicating that there might be other important functional traits, such as relative growth rate, wood density, and leaf phosphorous, aluminum and potassium concentrations which are suggested to be essential for microbial communities (Kembel & Mueller, 2014; Kembel et al., 2014), were not considered in our study. Note that comparing with previous studies (Laforest-Lapointe et al., 2016a,b, 2017a,b), the explanatory power of host species identity was low. One possible explanation for this discrepancy is that the stochastic processes in fragmented system has overwhelmed the effects of selection pressure of host species. In studies that conducted in natural continuous ecosystem, the host identity explained a much large fraction of variation in bacterial community structure [27% in Laforest-Lapointe et al. (2016a) and 47% in Laforest-Lapointe et al. (2016b)]. Another possible explanation is that previous studies have focused on a limited number of dominant plant species [five species for Laforest-Lapointe et al., (2016a,b); seven species for Laforest-Lapointe et al., (2017b)]. Our study sampled all tree species that occurred on the study islands, and therefore, included more variation in community composition. Among all island characteristic, we found among-island spatial distance explained the most variation in phyllosphere microbial communities. For fungal communities, the island spatial distance is a more important driver than host species characteristics (Table 4.1). Comparing with

bacteria, dispersal limitation is more important for shaping fungal communities, which was in line with previous empirical studies conducted across larger spatial scales (Barberán et al., 2015; Wang et al., 2017). This is probably due to the difference in the cell size of bacteria and fungi (Martiny et al., 2006), that is, the relatively smaller-sized bacteria are less restricted by long-distance dispersal than fungi (Fenchel et al., 1997).

Our study, to our knowledge, is the first to examine the driving factors of the structure of phyllosphere microbial communities in a fragmented ecosystem. We found that, unlike natural continuous ecosystems which were examined in previous phyllosphere microbial studies, the assembly of phyllosphere microbiome is highly stochastic. Comparing with island identity, host species identity explained more variation in the structure of phyllosphere bacterial communities. However, island identity explained more variation in phyllosphere fungal communities, with dispersal limitation among islands being the major factor. These findings improve our understanding of how habitat fragmentation alters the mechanisms determining the structure of plant-associated microbial communities. We also note that there is a large proportion of variation that was not explained by the factors we have examined. The ability of microorganisms to establish, thrive, and reproduce on the leaf surface depends on other factors such as microclimate, which exhibits fine-scale variations (Vacher et al., 2016). Even phyllosphere microbial communities of leaves from different parts of the same tree canopy may exhibit different structures (Leff et al., 2015). Future studies that involve individual- or leave-level plant traits, canopy structure, and microclimatic variables might be able to provide a more thorough understanding of the assembly mechanisms of phyllosphere microbiome.

APPENDIX A.

SUPPLEMENT TO CHAPTER 1

Table A. 1 – The values of the K statistic of species colonization and local extinction. Species extinction/colonization probabilities were defined as the proportion of plots in which a species went extinct/colonized within each treatment. Significant p values are shown in bold ($p < 0.05$).

	Colonization		Extinction	
	K	p	K	p
Control	0.043	0.95	0.124	0.04
N	0.248	0.01	0.061	0.54
Water	0.179	0.08	0.077	0.42
N+Water	0.081	0.45	0.112	0.29

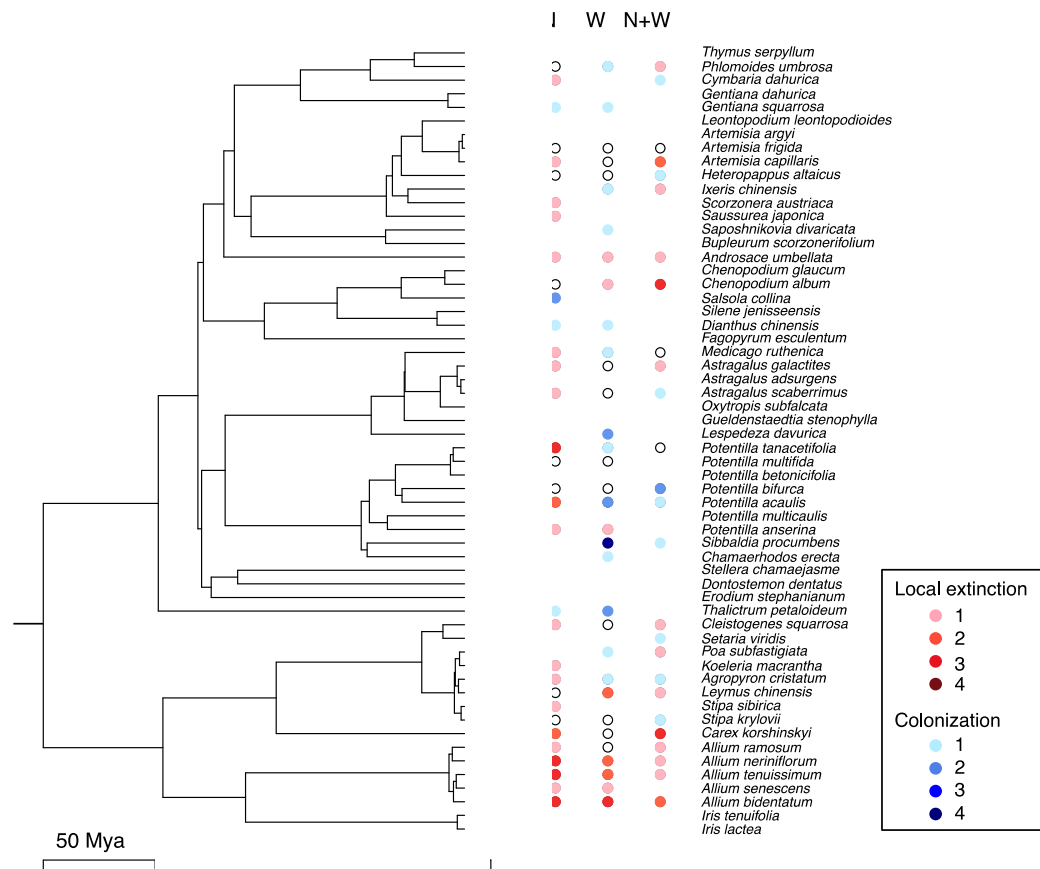


Figure A. 1 – Phylogenetic tree for the species that were observed in the experimental area and the phylogenetic distribution of species extinction and colonization. Points next to the phylogeny represent species that were locally extinct or colonists in different treatments. Different shades represent the number of replicate plots in which local extinction or colonization was observed. Open circles show the species that were present from 2005 to 2011 in all replicates.

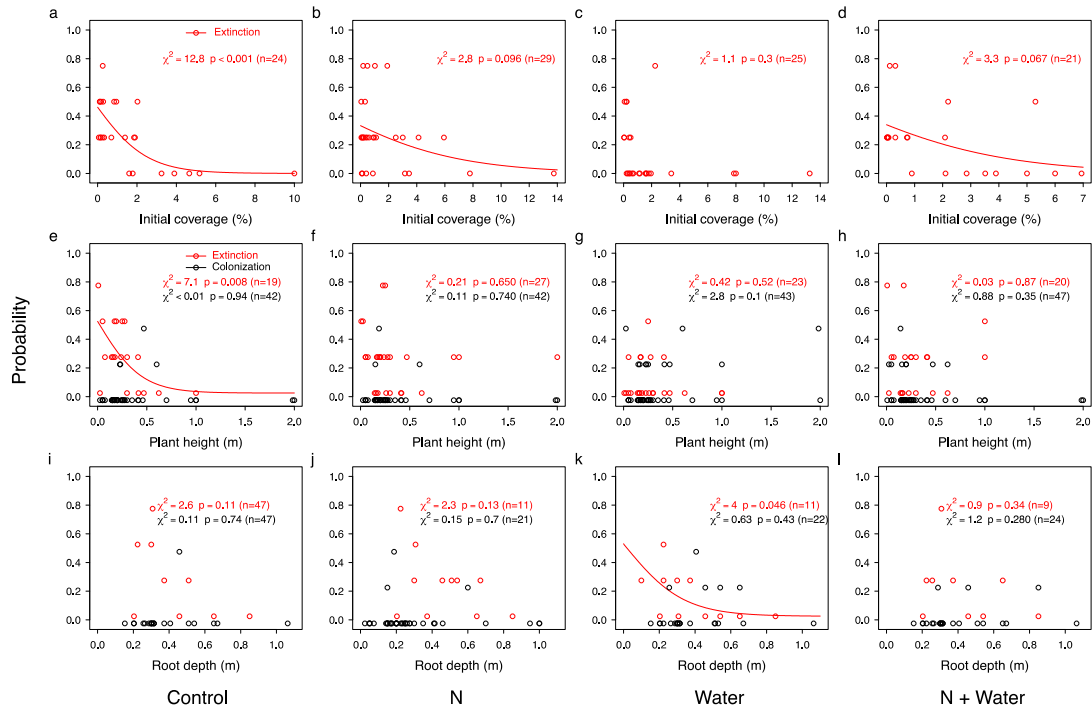


Figure A. 2 – Species colonization and local extinction as functions of initial abundance, plant height, and root depth in each treatment. Species extinction and colonization were defined as the proportion of replicates in which a species went extinct/colonized within each treatment. Significant logistic regression lines ($p < 0.1$) are shown.

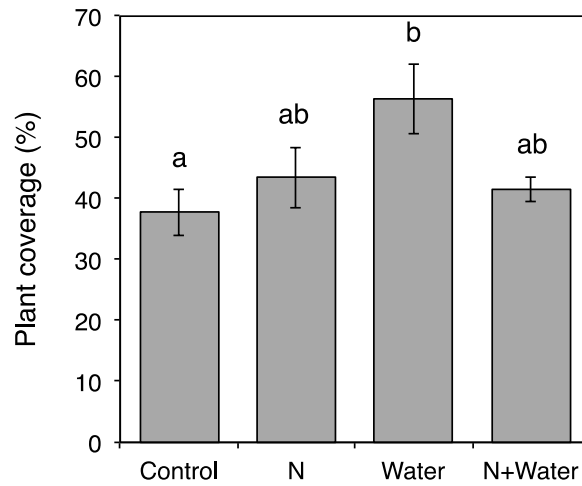


Figure A. 3 – Plant coverage in each treatment in 2011, the seventh year of the experiment. Error bars represent standard errors. Treatments sharing the same letters do not differ according to Tukey's HSD tests.

APPENDIX B.

SUPPLEMENT TO CHAPTER 2

B.1 Results on the functional trait dissimilarity ($SES.D_{pw}$) between colonists/extinct species of each plot and final species composition in the other three replicate plots within the same treatment

For species colonization, we found significant negative $SES.D_{pw}(C)$ for plant height in the controls (Figure B. 4a, one sample t-test, $p=0.006$), indicating that colonizing species in each control plot were more similar in height with species present in other replicate plots than expected by chance. For species extinction, we found significant positive $SES.D_{pw}(E)$ for SLA in the control and water addition treatments (Figure B. 4d, one sample t-test, $p<0.001$ and $p=0.012$ for control and water addition, respectively), indicating that extinction excluded species that were more different in SLA comparing with species in other replicate plots than expected by chance. In addition, in the N+water treatment, the $SES.D_{pw}(E)$ for plant height and rooting depth were significantly greater than zero (Figure B. 4b,f, one sample t-test, $p=0.022$ and 0.002 for plant height and rooting depth, respectively), indicating that extinction excluded species whose height and rooting depth were more different from species in other replicate plots than expected by chance.

B.2 Results on the relationship between species colonization/extinction and plant functional traits

In the control plots, plant height was a significant predictor of species colonization, where taller species tended to have a greater probability of colonization (Figure B. 5a). In the N addition plots, however, plant height was a significant predictor of species extinction, where species of shorter stature were more likely to go extinct (Figure B. 5b). In plots with both N and water addition, both plant height and rooting depth were significant predictors of species colonization, with short - statured and shallow-rooted species being more likely to colonize (Figure B. 5d,l).

Table B. 1 – Results of permutational analysis of multivariate dispersions (PERMDISP) on the effects of treatments on community taxonomic β -diversity (Bray-Curtis), phylogenetic β -diversity (D_{pw}), and their respective standard effect sizes (β -deviation and $SES.D_{pw}$).

	df	F	p value
Bray-Curtis	1	4.14	0.031
D_{pw}	1	3.46	0.051
β -deviation	1	2.83	0.083
$SES.D_{pw}$	1	5.31	0.015

Table B. 2 – Phylogenetic signal (Blomberg's K) of plant functional traits.

	K	p value
Plant height	0.045	0.895
Leaf N concentration	0.122	0.035
Specific leaf area	0.109	0.204
Rooting depth	0.079	0.458

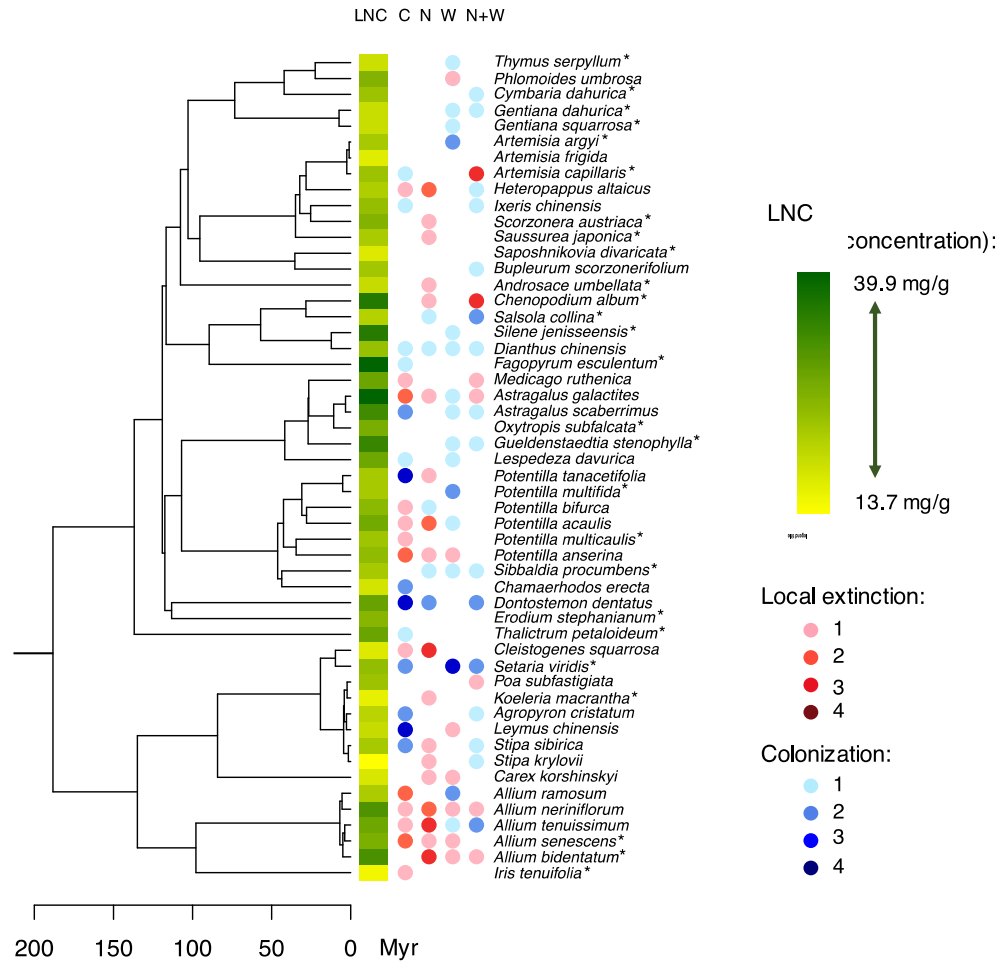


Figure B. 1 – Phylogenetic tree for the species that were observed in the experimental area. * denotes species whose rooting depth, leaf N concentration, and SLA were not directly measured and were instead extracted from the TRY database. The green bars next to the phylogeny represent leaf N concentration (LNC) of the species. Points represent species that were locally extinct (red) or colonists (blue) in different treatments. Different shades of the points represent the number of replicate plots in which local extinction or colonization was observed.

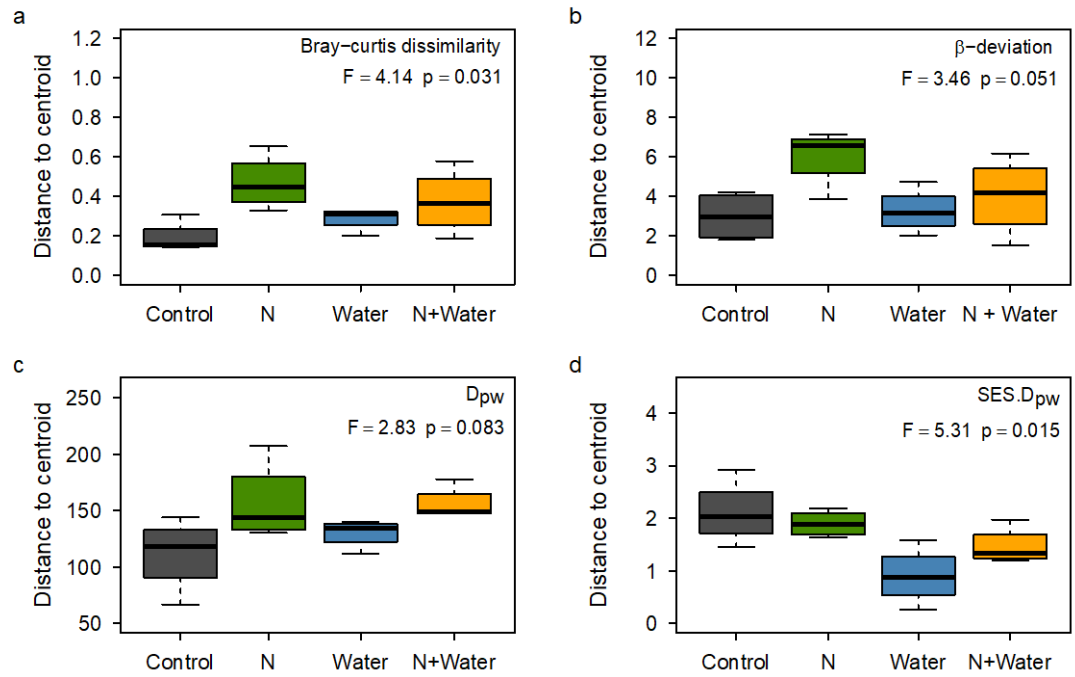


Figure B. 2 – Boxplot of multivariate homogeneity of groups' dispersion for each treatment in 2013. F and p values are based on permutation test for homogeneity of multivariate dispersions (PERMDISP).

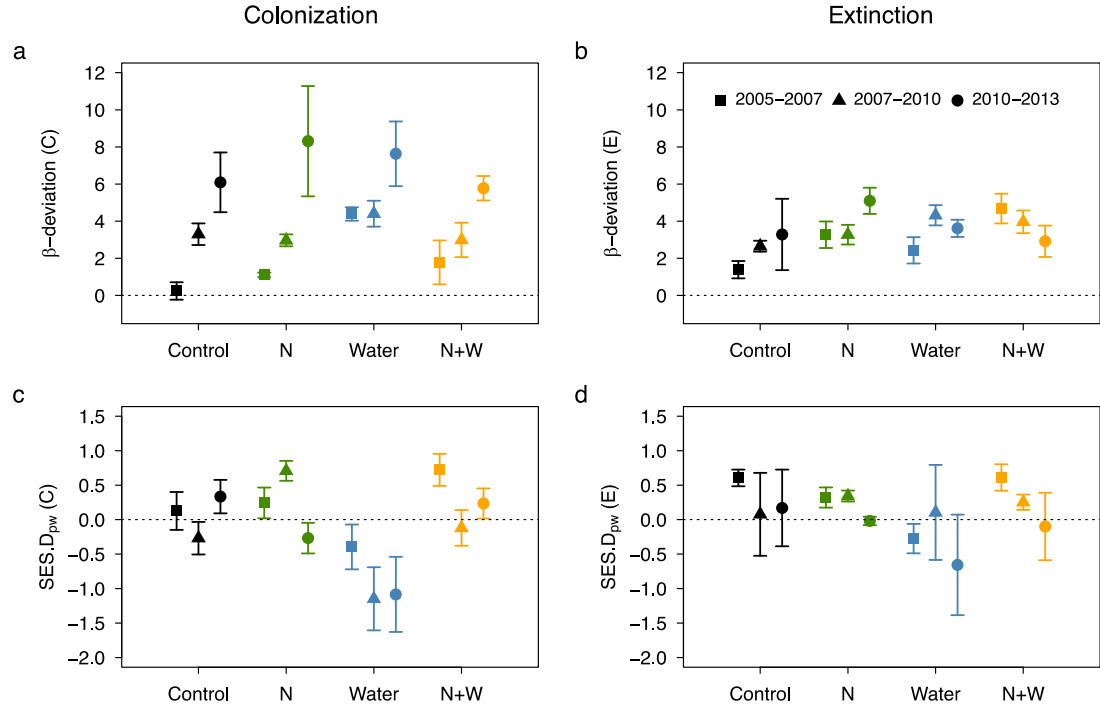


Figure B. 3 – The taxonomic (β -deviation) and phylogenetic (SES.D_{pw}) dissimilarity between colonists and extinct species of each plot and final species composition in the other three replicate plots within the same treatment over three time periods (2005-2007, 2007-2010, and 2010-2013). For species colonization, a negative β -deviation(C) indicates that new colonists are more similar to the final communities in other replicates than expected by chance, and a negative SES.D_{pw}(C) indicates that new colonists are more phylogenetically closely related to the final communities in other replicates than expected by chance, indicating deterministic colonization. For species extinction, a positive β -deviation(E) indicates that extinct species are more dissimilar to the remaining species in other replicates than expected by chance, and a positive SES.D_{pw}(E) indicates that extinct species are more phylogenetically distantly related to the remaining species in other replicates than expected by chance, indicating deterministic extinction.

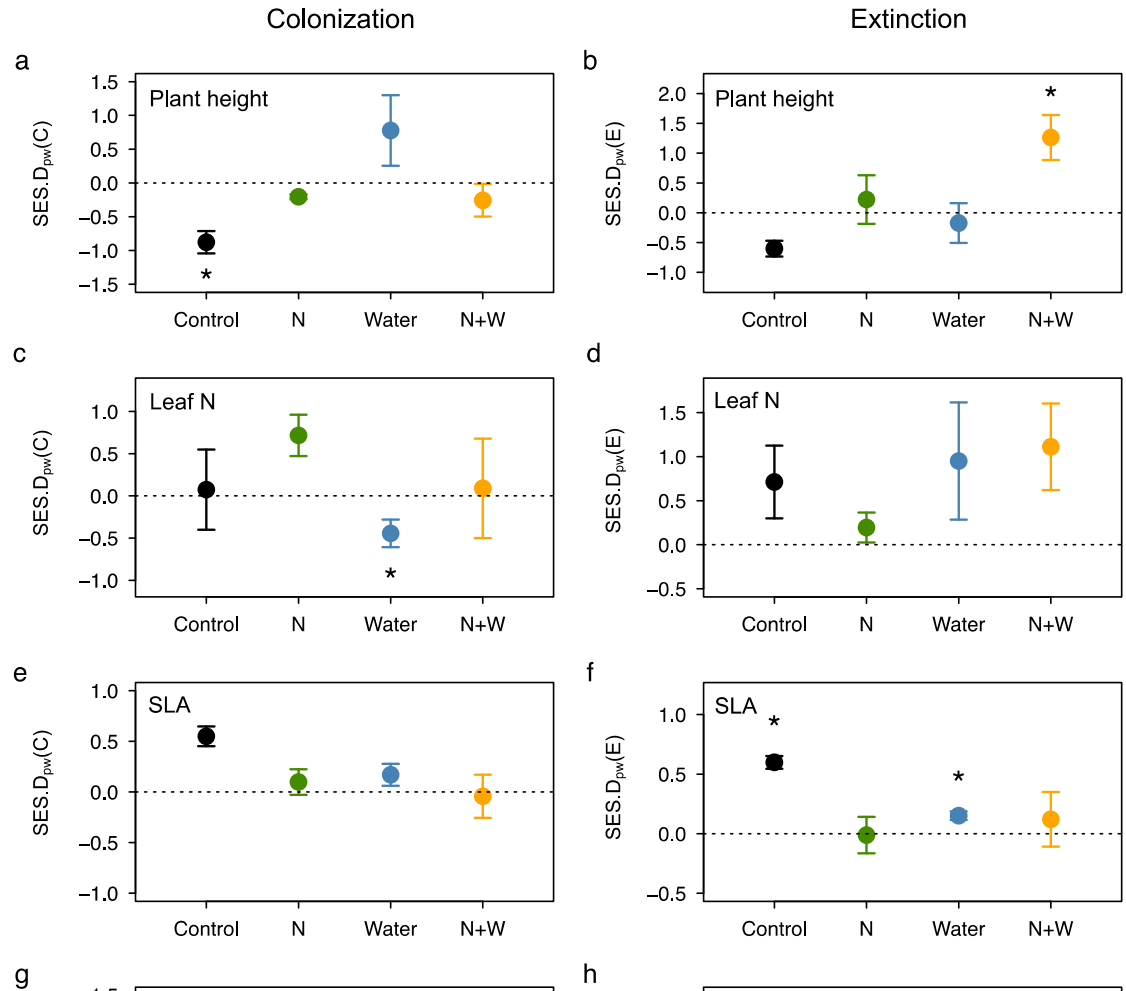


Figure B. 4 – The functional trait dissimilarity (SES.D_{pw}) between new colonists and extinct species of each plot and final species composition in the other three replicate plots within the same treatment. A negative SES.D_{pw}(C) indicates that new colonists are more similar to the final species composition than expected by chance. A positive SES.D_{pw}(E) indicates that extinct species are more different from the remaining species than expected by chance. * denotes values that are significantly different from zero based on one-sample t test (p < 0.05). Error bars represent standard errors.

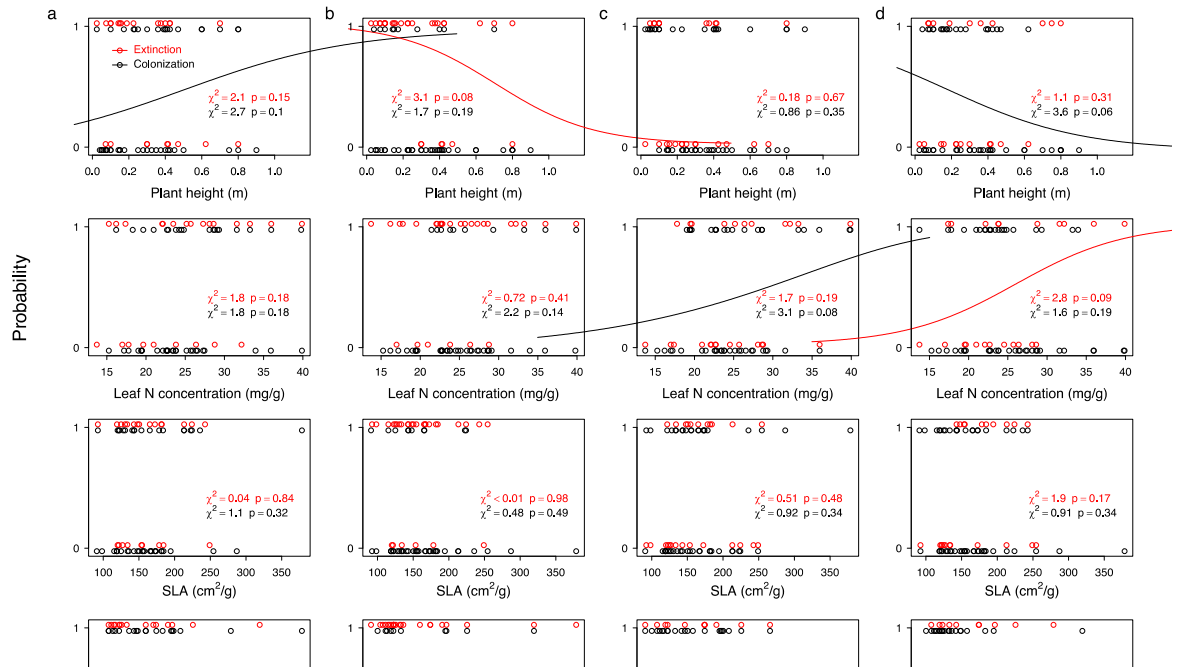


Figure B. 5 – Species colonization and local extinction as functions of plant height, specific leaf area (SLA), and root depth in each treatment. Species that did not colonize or go extinct in any plot within treatment was assigned a value of 0. Otherwise, species were assigned a value of 1. Significant logistic regression lines ($p < 0.1$) are shown.

APPENDIX C.

SUPPLEMENT TO CHAPTER 3

Table C. 1 – Results of ANOVA on the effects of heterogeneity loss, connectivity loss, and environment of the removed patch on changes in regional and local species richness. Changes in regional species diversity were quantified as the difference in γ -diversity between a metacommunity with patch removal and that of the control without patch removal. Changes in local species diversity were quantified as the difference in α -diversity between a local community in the metacommunity with patch removal and that of its counterpart in the corresponding metacommunity without patch removal.

			Regional diversity change		Local diversity change	
		df	F value	P	F value	P
(a) All	Heterogeneity loss (H)	1	45.053	<0.001	8.901	0.003
	Connectivity loss (C)	1	0.025	0.874	22.706	<0.001
	Environment of removed patch (E)	1	21.813	<0.001	48.655	<0.001
	H*C	1	3.328	0.071	1.622	0.204
	H*E	1	32.525	<0.001	26.783	<0.001
	C*E	1	3.155	0.078	18.275	<0.001
	H*C*E	1	8.475	0.004	12.354	<0.001
(b) Removing a light patch	Heterogeneity loss (H)	1	44.277	<0.001	33.392	<0.001
	Connectivity loss (C)	1	0.805	0.373	40.996	<0.001
	H*C	1	6.448	0.014	2.520	0.114
(c) Removing a dark patch	Heterogeneity loss (H)	1	1.233	0.271	2.394	0.124
	Connectivity loss (C)	1	4.209	0.044	0.120	0.730
	H*C	1	1.603	0.210	11.426	<0.001

Table C. 2 – Results of ANOVA on the effects of heterogeneity loss, connectivity loss, and environment of the removed patch on changes in regional and local ecosystem functions. Changes in regional biovolume production/seed decomposition were quantified as the difference in biovolume production/seed decomposition between a metacommunity with patch removal and that of the control without patch removal. Changes in local biovolume production/seed decomposition were quantified as the difference in biovolume production/seed decomposition between a local community in the metacommunity with patch removal and that of its counterpart in the corresponding metacommunity without patch removal.

		Regional					Local			
		Changes in biovolume production		Changes in seed decomposition			Changes in biovolume production		Changes in seed decomposition	
		df	F value	P	F value	P	F value	P	F value	P
(a) All	Heterogeneity loss (H)	1	0.677	0.412	10.921	0.001	0.167	0.683	6.835	0.009
	Connectivity loss (C)	1	0.076	0.784	1.348	0.248	1.038	0.309	1.293	0.256
	Environment of removed patch (E)	1	1.325	0.252	0.034	0.853	10.633	0.001	4.133	0.043
	H*C	1	2.866	0.093	0.790	0.375	0.022	0.883	0.046	0.830
	H*E	1	14.911	<0.001	10.964	0.001	41.442	<0.001	10.424	0.001
	C*E	1	0.148	0.702	0.260	0.611	0.644	0.423	0.443	0.506
	H*C*E	1	0.042	0.838	1.923	0.168	1.649	0.200	5.496	0.020

Table C. 2 Continued

(b) Removing a light patch	Heterogeneity loss (H)	1	9.656	0.003	18.014	<0.001	22.971	<0.001	14.809	<0.001
	Connectivity loss (C)	1	0.061	0.805	0.879	0.353	0.023	0.880	1.409	0.237
	H*C	1	1.362	0.248	2.001	0.163	0.634	0.427	1.968	0.162
(c) Removing a dark patch	Heterogeneity loss (H)	1	5.437	0.023	0.000	0.998	18.549	<0.001	0.223	0.638
	Connectivity loss (C)	1	0.033	0.857	0.234	0.630	1.692	0.195	0.131	0.717
	H*C	1	1.249	0.268	0.173	0.679	1.045	0.308	3.863	0.051

Table C. 3 – Results of ANOVA on the effects of heterogeneity loss, connectivity loss, and environment of the removed patch on changes in regional and local species richness after controlling for variation in species total abundance and biomass of the removed patch. Changes in regional species diversity were quantified as the difference in γ -diversity between a metacommunity with patch removal and that of the control without patch removal. Changes in local species diversity were quantified as the difference in α -diversity between a local community in the metacommunity with patch removal and that of its counterpart in the corresponding metacommunity without patch removal.

		df	Residual (Regional diversity change)		Residual (Local diversity change)	
			F value	P	F value	P
(a) All	Heterogeneity loss (H)	1	39.464	<0.001	10.562	0.001
	Connectivity loss (C)	1	0.097	0.756	20.550	<0.001
	Environment of removed patch (E)	1	24.579	<0.001	38.679	<0.001
	H*C	1	3.107	0.081	1.607	0.206
	H*E	1	33.092	<0.001	34.189	<0.001
	C*E	1	1.566	0.213	17.548	<0.001
	H*C*E	1	5.608	0.020	9.719	0.002
(b) Removing a light patch	Heterogeneity loss (H)	1	30.484	<0.001	25.207	<0.001
	Connectivity loss (C)	1	0.173	0.679	40.772	<0.001
	H*C	1	3.018	0.088	1.187	0.277
(c) Removing a dark patch	Heterogeneity loss (H)	1	1.412	0.239	2.436	0.120
	Connectivity loss (C)	1	6.382	0.014	0.018	0.895
	H*C	1	3.504	0.066	12.087	0.001

Table C. 4 – Results of ANOVA on the effects of heterogeneity loss, connectivity loss, and environment of the removed patch on changes in regional and local ecosystem functions after controlling for variation in species total abundance and biomass of the removed patch. Changes in regional biovolume production/seed decomposition were quantified as the difference in biovolume production/seed decomposition between a metacommunity with patch removal and that of the control without patch removal. Changes in local biovolume production/seed decomposition were quantified as the difference in biovolume production/seed decomposition between a local community in the metacommunity with patch removal and that of its counterpart in the corresponding metacommunity without patch removal.

		Regional					Local			
		Residual (Changes in biovolume production)			Residual (Changes in seed decomposition)		Residual (Changes in biovolume production)		Residual (Changes in seed decomposition)	
		df	F value	P	F value	P	F value	P	F value	P
(a) All	Heterogeneity loss (H)	1	0.114	0.736	6.418	0.013	0.150	0.699	5.382	0.021
	Connectivity loss (C)	1	0.017	0.896	1.007	0.318	0.259	0.611	0.693	0.406
	Environment of removed patch (E)	1	1.302	0.256	0.057	0.812	1.029	0.311	1.411	0.236
	H*C	1	2.544	0.113	0.593	0.443	0.017	0.896	0.073	0.788
	H*E	1	11.800	<0.001	7.211	0.008	37.355	<0.001	7.557	0.006
	C*E	1	0.159	0.691	0.009	0.926	0.023	0.879	0.752	0.387
	H*C*E	1	0.164	0.686	2.338	0.129	3.808	0.052	8.304	0.004

Table C. 4 Continued

(b) Removing a light patch	Heterogeneity loss (H)	1	1.977	0.165	2.680	0.108	14.903	<0.001	1.742	0.189
	Connectivity loss (C)	1	0.067	0.797	0.029	0.866	0.142	0.706	0.757	0.386
	H*C	1	2.099	0.153	2.060	0.157	1.811	0.180	4.565	0.034
(c) Removing a dark patch	Heterogeneity loss (H)	1	5.555	0.022	0.000	0.991	15.707	<0.001	0.485	0.487
	Connectivity loss (C)	1	0.059	0.810	0.139	0.711	0.435	0.511	0.260	0.611
	H*C	1	1.974	0.165	0.119	0.732	1.885	0.172	2.464	0.118

Figure C. 1 – Species density of each metacommunity and local community. (a) Species density of each metacommunity. (b) Species density of each local community. Bars with different colors represent different species. Bars in shade represent dark patches. Values are means \pm SE with density data measured as number of individuals per mL

(a)

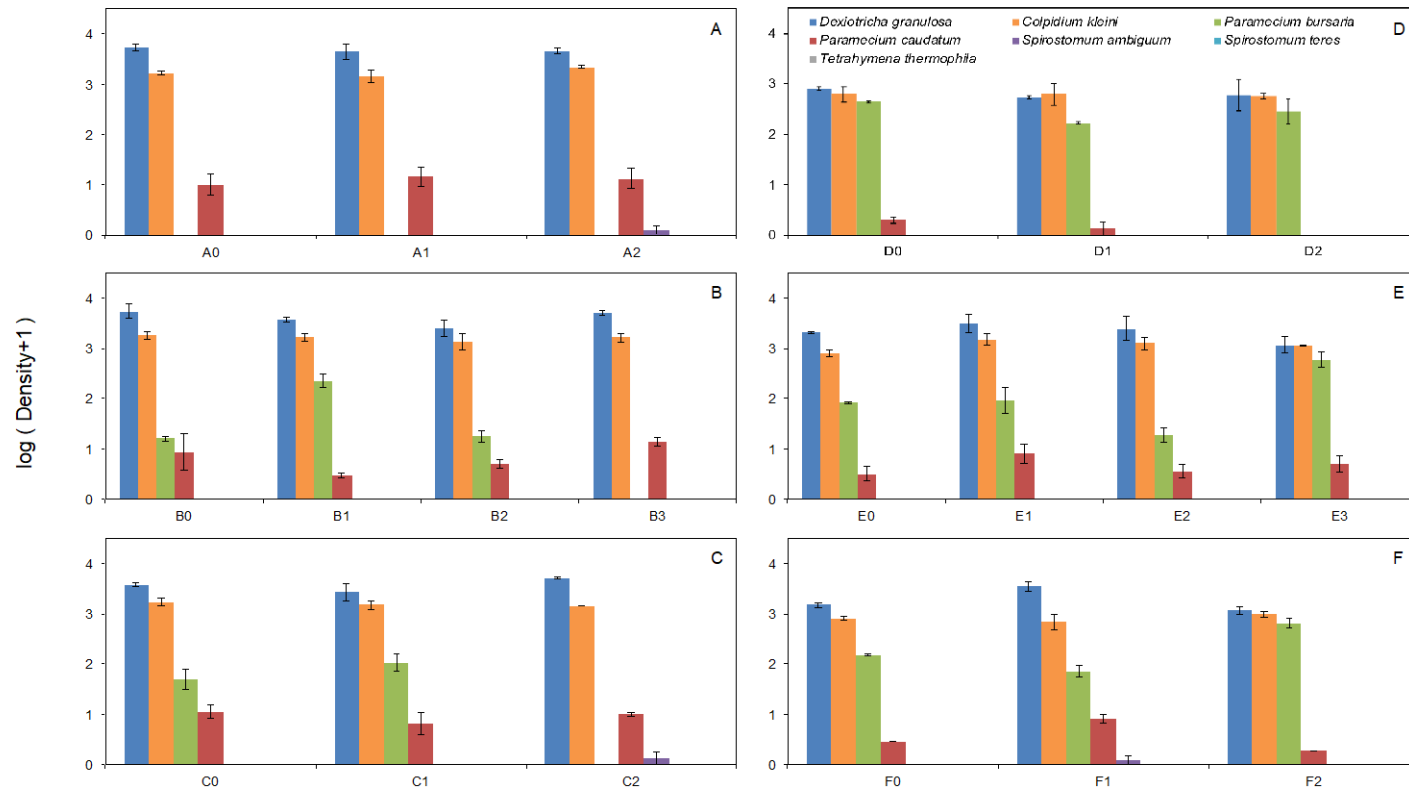


Figure C. 1 Continued

(b)

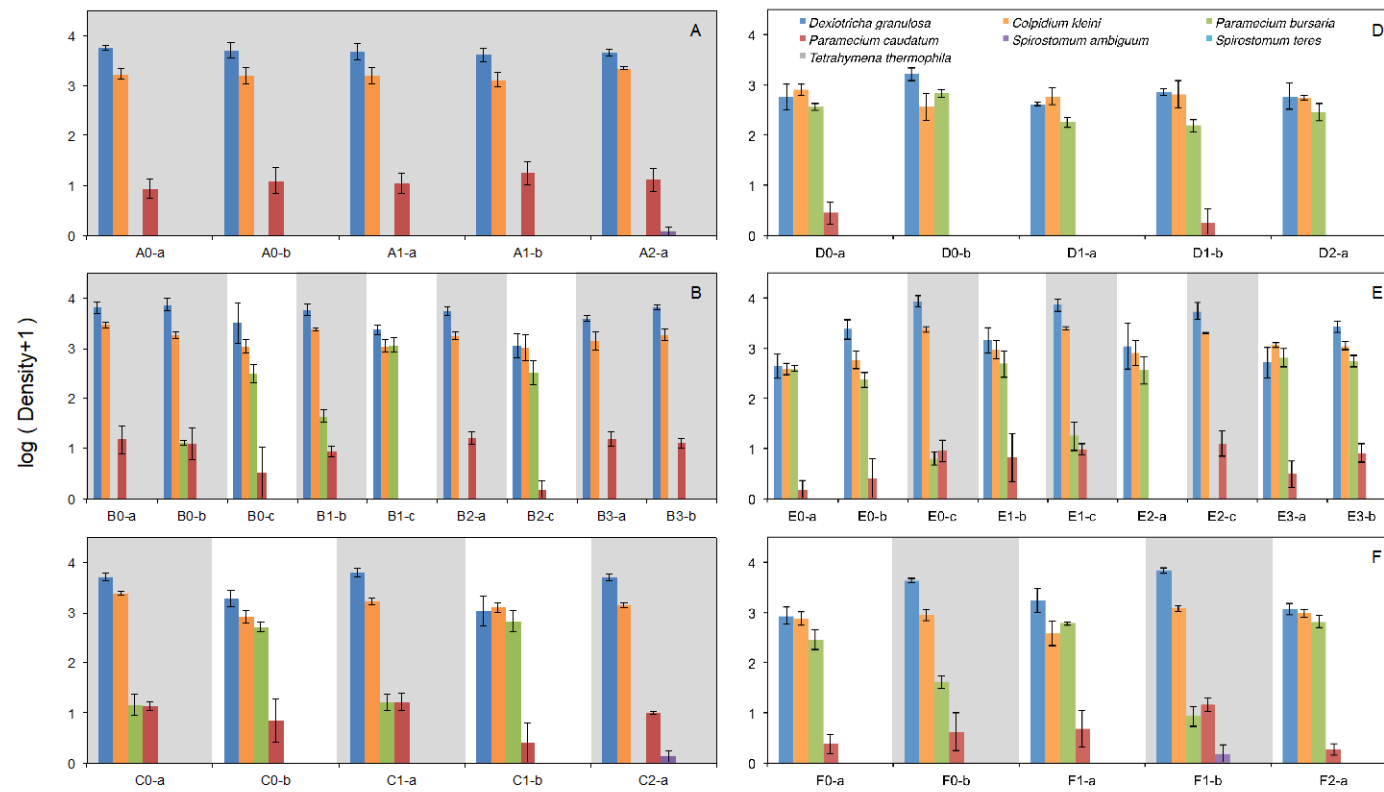


Figure C. 2 – Species biovolume of each metacommunity and local community. (a) Species biovolume of each metacommunity. (b) Species biovolume of each local community. Bars with different colors represent different species. Bars in shade represent dark patches. Values are means \pm SE.

(a)

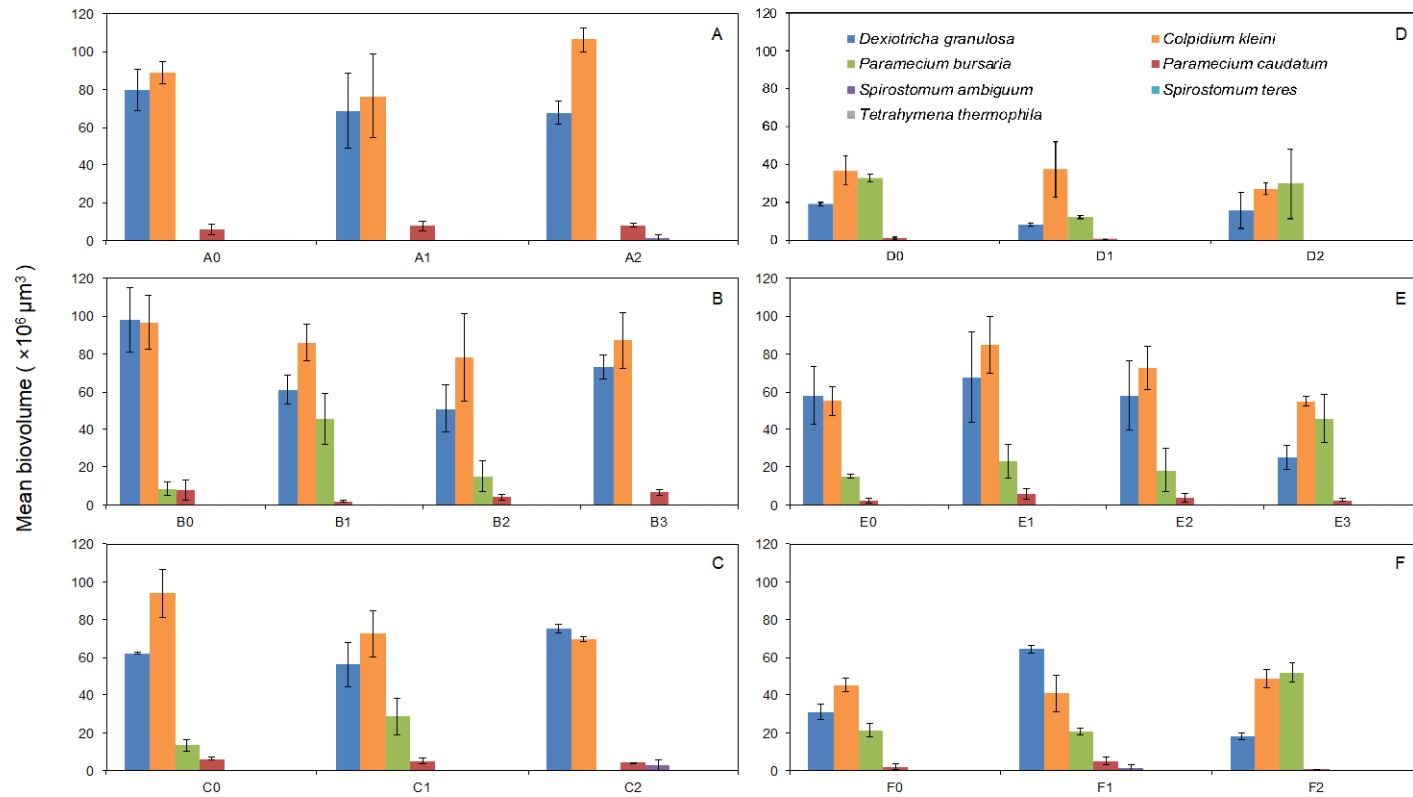
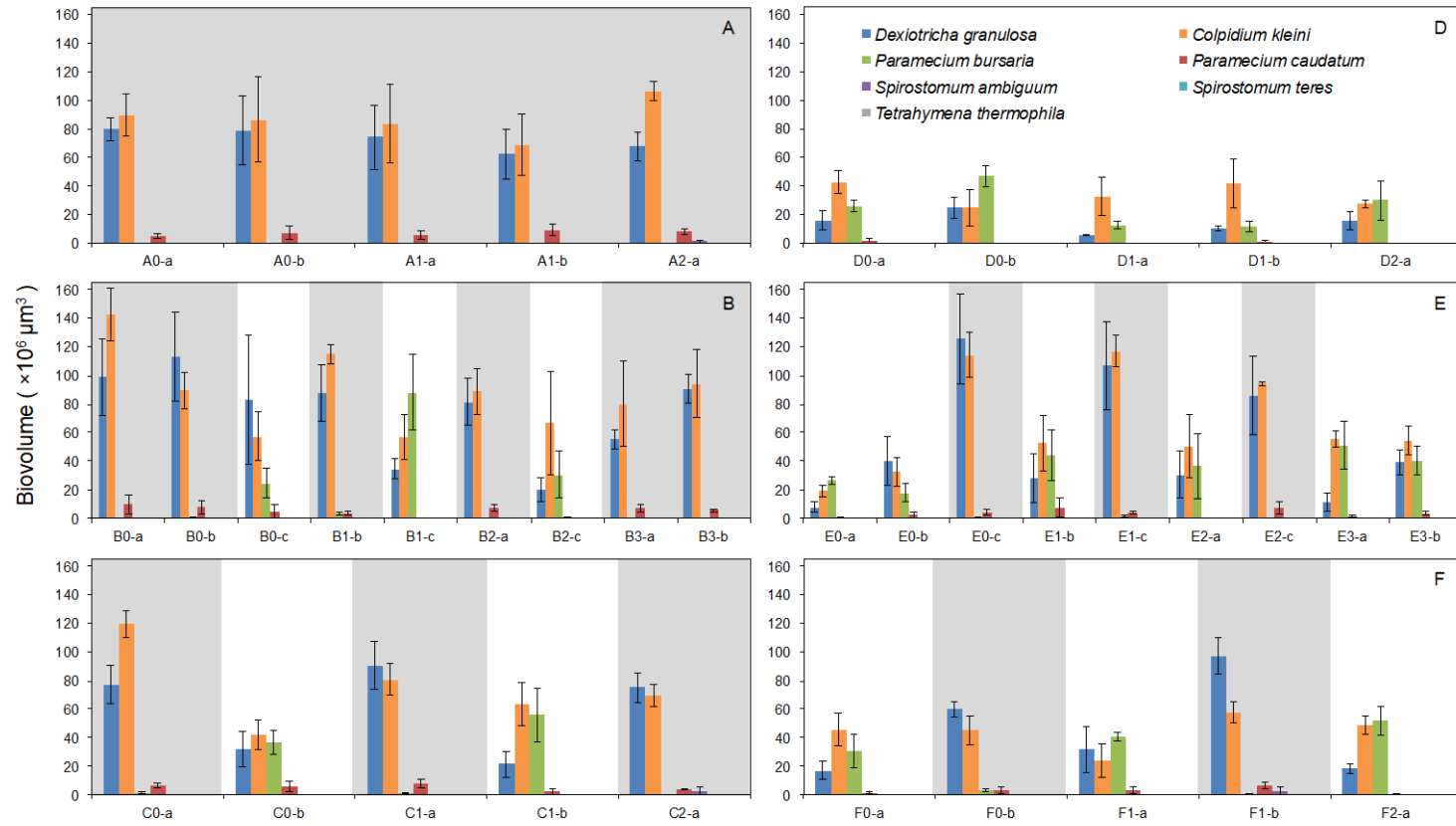


Figure C. 2 Continued

(b)



APPENDIX D. **SUPPLEMENT TO CHAPTER 4**

Table D. 1 – Area and geographical coordinates of the study islands.

Island	Latitude (N)	Longitude (E)	Area (hm ²)
I 1	29°30'53.72"	118°49'44.24"	9.7287
I 2	29°30'45.68"	118°49'26.12"	4.0584
I 3	29°30'24.09"	118°49'38.13"	3.0339
I 4	29°30'51.67"	118°49'27.67"	1.3589
I 5	29°30'15.06"	118°49'40.26"	0.9225
I 6	29°30'55.31"	118°49'20.37"	0.5279
I 7	29°30'49.27"	118°49'14.96"	0.4027
I 8	29°30'37.33"	118°48'56.19"	0.289
I 9	29°30'52.56"	118°49'21.63"	0.1888
I 10	29°30'51.40"	118°49'13.84"	0.0788

Table D. 2 – List of the plant species sampled on the study islands.

Species	Abbreviation	Family	Growth form	Shade tolerance
<i>Pinus massoniana</i>	PM	Pinaceae	Conifer	Light-demanding
<i>Cunninghamia Lanceolata</i>	CL	Taxodiaceae	Conifer	Light-demanding
<i>Juniperus formosana</i>	JF	Cupressaceae	Conifer	Light-demanding
<i>Liquidambar formosana</i>	LF	Hamamelidaceae	Deciduous	Light-demanding
<i>Quercus acutissima</i>	QA	Fagaceae	Deciduous	Light-demanding
<i>Quercus fabri</i>	QF	Fagaceae	Deciduous	Light-demanding
<i>Quercus serrata</i>	QS	Fagaceae	Deciduous	Light-demanding
<i>Rhus chinensis</i>	RC	Anacardiaceae	Deciduous	Light-demanding
<i>Symplocos paniculata</i>	SP	Symplocaceae	Deciduous	Light-demanding
<i>Albizia kalkora</i>	AK	Fabaceae	Deciduous	Shade-tolerant
<i>Dalbergia hupeana</i>	DH	Fabaceae	Deciduous	Shade-tolerant
<i>Diospyros kaki</i>	DK	Ebenaceae	Deciduous	Shade-tolerant
<i>Pyrus calleryana</i>	PC	Rosaceae	Deciduous	Shade-tolerant
<i>Ilex chinensis</i>	IC	Aquifoliaceae	Evergreen	Shade-tolerant
<i>Ilex rotunda</i>	IR	Aquifoliaceae	Evergreen	Shade-tolerant
<i>Castanopsis sclerophylla</i>	CS	Fagaceae	Evergreen	Shade-tolerant
<i>Cinnamomum camphora</i>	CC	Lauraceae	Evergreen	Shade-tolerant
<i>Cyclobalanopsis glauca</i>	CG	Fagaceae	Evergreen	Shade-tolerant
<i>Lithocarpus glaber</i>	LG	Fagaceae	Evergreen	Shade-tolerant
<i>Schima superb</i>	SS	Theaceae	Evergreen	Shade-tolerant
<i>Symplocos stellaris</i>	SY	Symplocaceae	Evergreen	Shade-tolerant

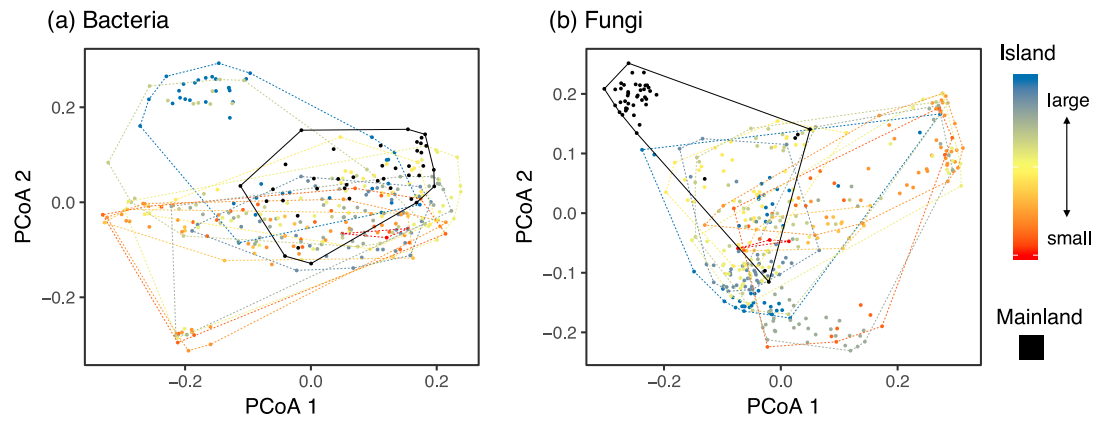


Figure D. 1 – Ordination of the phyllosphere bacterial and fungal community structures by principal coordinate analysis (PCoA). PCoA was performed based on pairwise Jaccard dissimilarity index. Samples are colored by island area, with black dots representing the samples from the mainland.

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